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RESEARCH, THE FRONTIER OF CIVILIZATION [1]

G. Fred Somers [2]

Research is defined by Webster as "Careful, systematic, patient study and investigation in some field of knowledge, undertaken to establish facts or principles."

Research then is a pushing back of the frontiers of knowledge. These frontiers are being pushed back at an accelerated rate all over the world. We are witnessing the unfolding of a new era—an era of science and technology such as was not even dreamed of only a few decades ago.

All of us recall from our studies of history the Industrial Revolution which began in Great Britain in the eighteenth century. While this "revolution" was not violent it nevertheless produced profound and far-reaching changes. The world and man's way of life were very greatly changed, but these changes were gradual and without social upheaval.

Now in the middle of the twentieth century we seem to be already in the first stages of a second industrial revolution. This one is founded upon a much more complex basis of science and technology, but its consequences promise to be just as profound and as far-reaching as the Industrial Revolution of the eighteenth century. Like the previous industrial revolution, it probably will not produce acute social upheaval, though no doubt the resulting social adjustments will be far-reaching.

The facts being brought to light by present-day science are important for themselves, and when they are applied effectively, when they are put to work, they become more valuable and powerful. It is important that all people, particularly those who would call themselves educated, be acquainted with the presence of this revolution and appreciate some of its likely consequences.

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[1] General Contribution No. 909. Read at the meeting of the Society for the Advancement of Research held in the U. P. College of Agriculture on February 24, 1959.

[2] Visiting Cornell professor of experiment station administration and biochemistry.

The evidences of this new revolution are all around us. Some are marvelous indeed. Witness the space vehicles: first, satellites around the earth; now, satellites around the sun and shots at the moon!

According to newspaper accounts Vanguard II or Alpha 1959 is predicted to last indefinitely as a satellite of the earth and to supply a daily record of cloud cover over a vast portion of the earth's surface.

Everyone is aware of the space vehicles, but little attention is paid to the complex machines necessary to launch and control them. Even less familiar are the equally complex machines—the high speed computers which have been compared to mechanical brains—which are necessary to control the flight of these rockets and to interpret the data supplied by the instruments on board them. The present revolution in technology would be impossible without such machines to handle rapidly all of the data required. These machines are finding even greater use in business, in research, and in the analysis of large masses of data of all kinds.

No less impressive but horrifying to contemplate, are the almost inconceivably powerful atomic weapons. It is difficult to imagine the destructive force equivalent to thousands, even millions, of tons of TNT exploding in a fraction of an instant.

Out of the research on atomic weapons has come atomic power to replace our fast dwindling resources of fossil fuels such as coal and oil. This can bring the blessings of adequate power resources to those nations not favored by fossil fuels and other sources of power.

Equally marvelous are the radio-isotopes made available by application of atomic energy. Many applications have been made already, and we have only begun. These include gages used in industrial processing for controlling the thickness of rubber sheeting in tire making and for packaging cigarettes, etc; irradiation of huge castings to reveal possible imperfections; monitoring of processes, such as the flow of oil or other fluids in pipelines; irradiation of plastics and metals to produce new properties; and preservation of foods.

It has been estimated that the application of radio-isotopes by U.S. industry alone will in a relatively short time, save more than

the two billion dollars ($2,000,000,000) expended in the initial development of atomic weapons.

There are many other applications of radio-isotopes. In medicine, one example is the use of radio-active iodine which can search out and report on the functioning of the thyroid gland. There is even some promise that this isotope might be used in therapy of thyroid disorders.

In agricultural research, radio-isotopes have been used in studies on the mechanism of ion uptake by plant roots; physiology of action of herbicides, insecticides and fungicides; production of new mutants as a source material for breeding new varieties; and in measuring how much of the added fertilizer is taken up by a crop.

These are only a few of the more striking advances in research and their application during the past few years.

The great bulk of our recent advances has resulted from more commonplace developments. New machinery, new chemicals for the control of pests and diseases, new varieties, and new production practices—all the products of research—have enabled man to produce more food and fiber with less labor than before. Moreover, these greater quantities of food can be delivered to the consumer more efficiently, in a form the consumer desires, and can still be more nutritious than that prepared by older methods.

Research has produced also the means for better transportation and communication. Because of the airplane and the radio, the Philippine Islands, for example, now have a far better chance to be really one nation than ever before. Now one can travel from Manila to Davao in two hours; can send a telegram to any city *in any island* in a matter of minutes. Think what this means in comparison to transportation and communication limited to the land and the sea.

Research has also produced luxuries—automobiles, radio and television, to mention three. Man is now freer than ever before from the limitations of his own environment and resources. In his own home he can enjoy realistic music of the masters; he can watch events in distant places.

What of the future? When one contemplates what research might do for the future he can be even more enthusiastic! The exploration of our earth is about complete in a geographic sense. Such frontiers of exploration that were the dreams and challenge of young

people in days gone by no longer exist. Research remains as the new frontier— the path to the unchallenged, the way to the unattainable, the means to the impossible. Out of research and its *intelligent application,* can come wonders almost beyond the imagination. If man does not destroy himself with avarice and greed, he can enjoy a better, longer, and happier life. There will be more and better food for everyone. Diseases will be controlled. Earth will no longer be the limit of man's thought and travels.

But all this will not merely *happen.* It must be *planned for,* and these plans must be carried out. There are a number of requirements which must be met if we, if you, are to reap this harvest of bounty. To some of these let us now turn our attention.

A greater social awareness of the needs and rewards of research must be cultivated. Research does not operate in a vacuum. Scientists need laboratories, they need experimental farms, they need equipment, they need supplies and, they need to be paid well enough to meet their needs for food, clothing and some of the luxuries of life. A scientist cannot be a research worker eight hours a day and do something else to provide sufficient income during the remaining hours of the day. Research workers need time to think about research. They cannot be fully productive if they must be concerned unduly with extraneous matters.

To meet all of these needs requires money. Money, which, in the final analysis, must come from our fellow citizens. Hence, we must keep them informed of our needs and help them to appreciate the role we play in benefiting them. Only under these circumstances can we expect their support.

By keeping the public informed of our accomplishments we can accomplish another more subtle goal. Probably every man has, to a greater or lesser degree, a need to be appreciated. He likes to feel that what he is doing is worthwhile. In a large measure such approbation can come, does come, from fellow scientists. But it can come also from the people we serve. Great is the satisfaction of that scientist who finds his research accepted and *used.* We in agriculture are particularly fortunate in having an extension arm to aid us in carrying our findings to the farm people. By using this arm effectively we can gain even greater financial support for our research, and I might add parenthetically that the needs of research workers for additional money seem never to be satisfied. But besides money to support our research we can gain that more intangible,

and possibly in some ways more important, satisfaction of being accepted, of being *valued.*

Another aspect of social conscience which needs to be developed is an atmosphere of freedom of inquiry. No problem, no area of ignorance, should be "out of bounds" for research. Each scientist must be free to investigate problems he deems important. The public may not always understand our actions. We must develop in them an attitude of tolerance and confidence. This is not always easy. Some attitudes on the part of scientists can help. For example, we must not assume that a freedom of inquiry gives us a license for irresponsibility. Our support comes from society and we have a responsibility to that society. Particularly in a public-supported institution, scientists should identify their research with the goals and needs of society. More important, these needs must not be merely *guessed* about. We must gain first-hand familiarity with the problems and with the people who have problems. Only then can we evaluate our research programs in the light of the problems which need solution.

Another aspect of the future of science is definitely a public concern. The shortage of scientific manpower throughout the world is causing grave concern. More young people must be encouraged to follow the path of science. To do so will undoubtedly require an improvement in our schools and in our attitudes toward education. Training must extend beyond the B.S. degree. Even the Ph.D. degree is hardly sufficient in some areas of scientific endeavor. The Philippines as a nation needs to face this problem squarely; to provide the means for a greater proportion of its young people to seek higher education. Greater attention must be given to providing training beyond the B.S. degree at universities in the Philippines, and this training must be rigorous and realistic.

Schooling alone, however, is not enough to make a scientist. Added to this must be a genuine devotion to a search for knowledge—a curiosity without bounds. Only those with a genuine intellectual curiosity will ever pierce the walls of ignorance. Such an attitude cannot be developed by one individual alone. It must be developed from youth, and must be encouraged and nurtured even in mature scientists. It finds its greatest stimulus in a society that is intellectually curious. Such an attitude can be developed, but all too often it is only a haphazard by-product, rather than one of the main aims of our educational processes. Too often students are allowed to be satisfied with merely passing courses. Too often is the assump-

tion made that the only way to learning is to "take a course." One of the great virtues of Oxford University is that it expects its young scholars to search out knowledge on their own outside and beyond the limits of the course program. At that university it is not, nor anywhere else should it be, acceptable to excuse ignorance of a subject by saying "I have never had a course in that." The wisdom of mankind is preserved in our libraries. Students must be taught to turn there for the information they need or desire. Failing to find it there they should learn that there still remains the avenue of research. Unless the habit of depending more upon their own investigation and less upon formal course work is well developed, no student can ever become a first-rate scientist. Unless a student is taught to read, to evaluate, to correlate beyond his course work while still a student, it is unlikely that he will ever do it to the degree required for first-rate research. Teaching the *methods* of science probably is far more important than teaching the findings of science, particularly for advanced students. These methods are more subtle; less easy to teach.

Schooling and a thirst for knowledge by themselves are still not sufficient. Coupled with these must be honesty, integrity and a *willingness* to work. A scientist must be able to face facts honestly and squarely. To evade, to rationalize, to substitute wishful thinking for honest, critical, analytical evaluation is worse than useless. It can be dangerous. Nothing short of truly objective evaluation of research data should be sufficient. One must always be prepared to re-examine and discard his evaluations and hypothesis if further evidence requires it. This applies to scientists of all ages. To be less than objective can be unfortunate in the case of young scientists. It can be seriously detrimental in the case of older scientists. One could cite many cases where the progress of science was seriously retarded when a senior scientist refused to accept the findings of a younger man because it failed to conform to his hypothesis. Let me cite one example.

"In 1886, MacMunn discovered a pigment in muscle and other animal tissues. He found that this pigment was an iron porphyrin and called it 'myohematin' or 'histohematin.' His work was so harshly criticized by Hoppe-Seyler and his pupil Levy that MacMunn abandoned his research with myohematin altogether, and scientists assumed that the compound discovered by MacMunn was merely a mixture of decomposition products of hemoglobin.

"In 1925, Keilin showed that MacMunn was right and that myohematin is a distinct compound present in tissues." [3] This is the compound now called cytochrome and recognized to be an essential component of the respiratory mechanisms of most aerobic organisms. An appreciation of MacMunn's finding was delayed for 40 years because it didn't conform to the pre-conceived notion of an influential scientist. Let us use our influence cautiously, particularly when we are tempted to disregard or depreciate new findings!

A research worker must be willing to work. This is important. Even the best ideas come to naught unless they are developed, unless their consequences are explored. To do so requires long, patient and careful work. It has been said that "genius is one per cent inspiration and 99 per cent perspiration." If I were to find a fault with this generalization it would be that it puts too *little* emphasis upon *work;* too much emphasis on inspiration. Not all of us in our lifetime will come up with even one revolutionary new idea, but most of us, if we are willing to work, can nevertheless move the cause of science ahead. Moreover, it is familiarity with a problem which stimulates new ideas. I have often said that you cannot give too good a man the task of helping to collect even routine data. I doubt if any scientist is too good, too valuable to be present when the basic data, routine though they be, are being taken on his experiments. Frequently, little irregularities, things that are not explained, are apparent at such a time. To the routine investigator, to one without a trained curiosity, such things are merely a nuisance; often passed off as just "one of those things." To the trained observer, to the man with a trained curiosity, these little anomalies are frequently the clue to something new. Many times they lead to something important.

Let me illustrate. For a few short years I was fortunate to have the opportunity to work with the late J. B. Sumner. In 1926, he was studying the enzyme urease. One afternoon he placed an aqueous acetone extract of jack bean meal in a refrigerator and left it overnight. (He was an ardent tennis player and I suspect the time for tennis came before he had completed the extraction.) Actually the extract he placed in the refrigerator was not complete. It was still filtering; so he put filter funnel, receiver and all into the cold.

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[3] J. B. Sumner and G. F. Somers, "Chemistry and Method of Enzymes" 3rd ed., Academic Press, New York, 1953. p. 227.

The next morning he observed something white at the bottom of the cylinder into which the extract had filtered. Those of you who have worked with plant extracts, especially those high in protein content, will recognize that this is a common occurrence. Precipitates frequently appear, and usually they have no great significance. Sumner, however, had the curiosity to examine this material. Lo and behold, he found *crystals* in it! His curiosity led him on to determine what they were. Much additional, patient, and tedious research established that these crystals, which most men would have ignored, were actually crystals of the *enzyme urease!* He had prepared the *first crystals* of *any enzyme.* It took years for the skeptical scientific world to accept his evaluation of his finding, largely because it did *not* agree with the previous notions of Willstatter in Germany, who was at the time the world's foremost authority on enzymes. Willstatter was eventually proven to be wrong and for his momentous finding Sumner shared the Nobel Prize for Chemistry nearly 20 years later. He, with the help of others, finally won, over the opposition of the reputable Willstatter, but the latter undoubtedly delayed the progress of enzyme chemistry for years because of his influence.

A research worker must develop a sense of responsibility. I have mentioned earlier the need to have a sense of responsibility to the public—to identify and solve the problems of the people from whom we receive support. We must do more than this. Research must be kept in balance. To quote an analogy from Woodrow Wilson: "Not all change is progress, not all growth is the manifestation of life. Let one part of the body outgrow the rest and you have malignant disease, the threat of death." In a similar way we have a responsibility to see to it that all aspects of a problem are developed, to see to it that all areas of research receive support. There is little purpose in developing better varieties of rice, for example, if the seeds of these varieties are not made available to farmers. Even if the seed is made available to the farmer, he may benefit little if he does not have additional information on fertilizers, disease control, insect control, when to plant, etc., to aid him in realizing the full potential of his new crop. Furthermore, it may benefit him little even if he grows a bumper crop, if he cannot market the additional produce advantageously.

I should hasten to add that research should be kept in balance by pushing along the *slow* rather than holding back the fast. In this case, administrators, good ones that is, have a very definite role to play.

In addition to these responsibilities there are others that might be mentioned. Each scientist has a responsibility to his organization. He should strive to make it the best in the country. This means rising above petty jealousies which can stifle cooperation and crush morale. It means anticipating the desires and needs of others and assisting them in working toward the common goal.

We as scientists have public responsibility of another kind. There are cases where the application of research can have far-reaching consequences. We have a responsibility to make such consequences known. For example, improvements in technology should — in fact, can be expected to lead to a decrease in the number of workers required on the farms. This is particularly true in the Philippines. The public needs to be aware of this and to capitalize on the consequences. This will mean more people available for employment in factories and business. Provision must be made to put these resources of human labor to productive use. To do otherwise will lead to chaos. The forces are being unleashed at the moment and their effect will grow with the years. The seeds of social unrest have been laid and unless there is a greater willingness among those who have capital and who could invest in the factories needed to utilize productively this inevitable surplus of farm labor, chaos can result. As intelligent scientists, as responsible citizens, can we afford to stand idly by? Should we not be heard?

I have outlined a vigorous program of preparation, work and responsibility for the research worker. To those of you who are still students the question may occur. "Is it worthwhile? What can I gain by choosing to be a scientist?" There is no simple answer to such questions. Probably one of the greatest rewards is the feeling of accomplishment. It is a great satisfaction to be able to say "This is what *I* did!" It is a great satisfaction to see the germ of an idea grow into full flower, to see your own creations, as it were, come into being. Moreover, you can expect to be paid well, but not richly, for doing in a large measure just about anything you want to do. After all no one can really tell the mature, capable scientist what to do and how to do it. If he is worthy to be classed as a scientist he must of necessity be afforded a large measure of freedom. There are few endeavors in which a man can be as free as he can in research work and at the same time be paid well for his efforts.

There is another aspect of research that I think worthy of mention. It seems to me that the attitudes and training required for research have a far deeper meaning than merely satisfying man's

curiosity and supplying his physical wants. Research to be effective must strive for objectivity. Everything must be judged by facts and such facts have no respect for persons, status or opinions. The truth of the matter is that experimental results *frequently* do not conform with the tentative hypothesis one has developed. One is then forced to re-evaluate, to once again ask a question, to search again for the truth. Each time he comes closer to a full understanding. Always, however, there are unanswered questions. Each answer opens new avenues for inquiry and we progress as rapidly as we ask intelligent questions, as rapidly as we adjust our thinking to the facts.

I suggest that if this same process were applied to our public affairs we could progress much faster toward a real understanding among individuals and nations.

One hears much these days about liberal education which means, as I understand it, gaining an appreciation of man as a social being; gaining perspective with respect to man's accomplishments and weaknesses; and gaining some insight into the philosophies which have guided societies through the centuries. It seems evident that, properly taught, the concepts basic to good research work can contribute to a liberal education. The basic assumption of the research approach to the solution of problems is an optimistic one, namely, that all problems can be solved. Some may take a long time, but nevertheless, they will ultimately yield to man's efforts. This then provides a significant purpose to the activities of those engaged in research. It provides a force to counteract the fatalism which says that the lot of mankind is hard, was meant to be, always has been, and always will be. Research provides the means, essentially the only means, for man to improve his material lot.

The objectivity of science; the critical, patient search for the facts; and the analytical, objective evaluation of these facts are values worth teaching for themselves. More than attitudes are involved. Our civilization is now based on research as no civilization was before. That portion of the world society which makes the most effective use of the findings of research will advance civilization fastest. Only an informed and appreciative society can do this and hence, in the same way as we teach other values in our schools we should teach, even to non-science students, the values of research.

It is important that the future lawyers, businessmen, politicians, housewives, laborers, every citizen, have some appreciation of such a basic force in our present civilization.

ALUMINUM AND ITS POSSIBLE RELATIONSHIP TO THE CADANG-CADANG OF COCONUT [1]

J. R. Velasco, A. Holaso, R. S. de la Peña,
E. Pantastico and V. F. Guevara

WITH THIRTEEN TEXT FIGURES

This paper is a report of further studies on soil factors as possible cause or causes of the cadang-cadang of coconut. Chemical analyses showed among other things that available aluminum in College soil was 0.015 per cent; in Pili soil, 0.043 per cent; and in Nasisi soil, 0.052 per cent. Because there were three to three and one-half times as much available aluminum in the cadang-cadang-affected soil (Pili and Nasisi) as in the non-cadang-cadang soil (College), toxic level of aluminum was suspected as one cause of cadang-cadang. Soil and solution culture studies seemed to confirm this suspicion. With three plant species, the aluminum cation was found toxic even at 2.5 ppm in the culture solution, especially under acidic conditions. The cation was more toxic than the aluminate anion.

Based on these and previous observations, it was postulated that cadang-cadang of coconut may be caused by toxic levels of aluminum which adversely affect the water economy of the palm thus bringing about the progressive diminution of the crown of leaves. The severe premature fall of nuts and the ultimate inhibition of the fruit bunches may be due in part to low levels of copper.

In a previous paper (Velasco, 1957) indications which pointed to some disturbing factor or factors in the soil as the probable cause of cadang-cadang were reported. Data were adduced to show that copper content of soil samples gathered from the Bicol Region was low relative to soil samples from cadang-cadang-free groves in Laguna and Quezon.

However, subsequent exploratory trials on the possible fertilizing value of copper sulfate, either added to the soil or sprayed to the plants growing in Bicol soil, yielded erratic results. The premature fall of nuts seemed to have been minimized by application of copper sulfate solution in the clasping leaf bases of coconut, but the growth of other plant species treated with copper sulfate did not consistently improve. This may mean that either copper deficiency was not

[1] Central Experiment Station Contribution No. 2005. Part of research project No. 1097. The writers are grateful to Dr. G. Fred Somers, Cornell Visiting Professor, and Dean L. B. Uichanco for various suggestions on the manuscript.

critical, that the mode and form of application was not suited to the plants, or some detrimental factor offsets whatever beneficial effect may accrue from the application of copper.

The present paper is a report on further studies on the problem based on analysis of the soil. To test those factors indicated by this analysis corollary experiments were started in the coconut groves of Pili, Camarines Sur, and Nasisi, Ligao, Albay, as well as in the greenhouse and experimental yard of the College of Agriculture. Occasional trips to the Bicol Region afforded the writers incidental opportunities to make further general observations which helped crystallize certain ideas and hypotheses. The work covered a period of approximately two years (August, 1957, to June, 1959).

SOIL ANALYSIS

Chemical analysis revolved around the assumption that whatever deleterious factor is present in the soil should be manifested either as a constituent which is peculiar to the Bicol soil samples or as one which is conspicuously high in these samples. The work proceeded along two complementary lines, namely, qualitative and quantitative analyses.

Qualitative analysis. Because the work was preliminary, only three samples were used: from Nasisi, Ligao, Albay; Regional Agricultural School, Pili, Camarines Sur; and College of Agriculture, Los Baños, Laguna. This narrow approach was thought expedient as an initial step because too much work would be involved if a more representative sampling is attempted.

All the soil samples were composited from several samples obtained by making random borings with the soil auger up to a depth of six inches. The field from which the Nasisi sample was taken was about one hectare located beside a stream and was apparently an alluvial deposit. The trees were ten to fifteen meters tall and in various stages of cadang-cadang decline. The Pili sample was obtained from one hectare of well-drained, rolling area. The cadang-cadang-infested trees were about five meters tall. The College sample was obtained from a grove near the Higamot Hill in the Central Experiment Station. The trees were about twenty meters tall, healthy and heavily fruiting. All the samples were air-dried and pulverized. They were then dried in the oven at 100°C and screened through a 40-mesh sieve.

Among the methods tried in obtaining extracts from the soil were sodium carbonate fusion, sodium peroxide fusion and digestion with *aqua regia*. After preliminary evaluation, the last method was decided upon. The silica was removed from the extract by digestion with hydrochloric acid to avoid interference from colloidal silicates in the qualitative tests. Ions were grouped according to Noyes' qualitative scheme (Noyes, 1922) before individual elements were analyzed. Table 1 shows the results of analysis. Copper was

TABLE 1.—*Qualitative analysis of the aqua regia extract of three soil samples*

ELEMENT	TEST REAGENT	COLLEGE	PILI	NASISI
Silver	Dithizone	—	—	—
Mercury	Dithizone	—	—	—
Lead	Dithizone	—	—	—
"	Potassium dichromate	—	—	—
Bismuth	Alkaline stannite solution	—	—	—
Copper	Carbamate	+	?	—
Cadmium	Hydrogen sulfide (yellow Ppt may be sulfur)	—	—	—
Arsenic	Stannous chloride	—	—	—
"	Silver nitrate	—	—	—
Antimony	Brilliant green	—	—	—
"	Phosphololybdic acid	—	—	—
Tin	Ammonium molybdate	+	+	+
Molybdenum	KCNS plus sodium thiosulfate	—	—	—
Aluminum	Aluminon	+	+	+
Cobalt	Ammonium thiocyanate plus acetone	—	—	—
Nickel	Dimethylglyoxime	—	—	—
Chromium	Diphenylcarbazide	—	—	—
"	Hydrogen peroxide	—	—	—
Zinc	Dithizone	+	+	+
"	Resorcinol	—	?	+
Manganese	Sodium bismuthate	+	+	+
"	Potassium periodate	+	+	+
Iron	Potassium ferrocyanide	+	+	+
"	Potassium thiocyanate	+	+	+

demonstrated in the College sample but was not definitely demonstrated in the two samples from the Bicol region. Tin, aluminum, zinc, manganese and iron were present in all the three samples.

Since there was not any non-essential element which characteristically set apart the Bicol samples from the College sample it may be postulated that cadang-cadang was not caused by a toxic substance peculiar to Bicol soils; or, if such a substance were present at all, it must be in so minute an amount as to escape detection by ordinary

qualitative chemical analysis. If the latter alternative holds then the element must be so potent that it could still inflict damage to the plants in spite of its small quantity. This possibility, which has a rather low probability will be explored when other approaches prove futile.

Quantitative analysis. In this study, the amount extracted by Morgan's sodium acetate-acetic acid solution (Peech and English, 1944) was determined and arbitrarily considered as available. Results of analysis for available manganese, iron and aluminum contents of the three samples are shown in table 2. Pili soil had

TABLE 2.—*Quantitative analysis of the Morgan's extract of the three soil samples*

CONSTITUENT [a]	COLLEGE SOIL	PILI SOIL	NASISI SOIL
Manganese, per cent	0.0020	0.0068	0.0012
Iron, per cent	0.00024	0.00066	0.00051
Aluminum, per cent	0.015	0.043	0.052

[a] Sodium bismuthate was used for determining manganese, O-phenanthroline for iron and aluminon for aluminum.

more than thrice as much manganese as College soil, while Nasisi soil had about one-half as much. Manganese was eliminated as a probable cause of cadang-cadang because the high levels of manganese were not consistently associated with the occurrence of the disease. However, its relatively high content in Pili soil may be one factor which contributes to the generally poor performance of coconut in that area.

Pili soil contained about 2½ times and Nasisi, about twice as much iron as College soil. Since the levels of iron even in Pili soil which contained the highest are quite low, this element may not be suspected of exerting toxic effects.

Aluminum in the cadang-cadang soils was from 2½ to 3 times as much as in College soils. Nasisi soil, which contained the highest, had 0.052 per cent or 520 parts per million (ppm) of available aluminum. In Hoagland's culture solution (*ca.*pH 4.8) 2.5 ppm of aluminum as aluminum sulfate was toxic to tomatoes.

Fresh Mayon Volcano ash was analyzed by Galvez (1938) and was found to contain 19.37 per cent total alumina (Al_2O_3). It had a pH value of 5.10 as against pH 6.26 for the surface soil in the vicinity. Recognizing that the total amount of an element in the soil may give a misleading impression of the fraction effective on plants,

Galvez, Aquino and Mamisao (1939) determined what the soil held in reserve (i.e., the amount extractable by 10 per cent HCl). This presumably includes the form ultimately available to plants after sufficient weathering. Among other things they found that the reserve alumina in the fine ejecta of Mayon Volcano was 4.67 per cent; in the lava, 3.32; in the surface soil, 8.08; and in the subsoil, 10.15. It may be of interest to note that Galvez (1936) found in upland soil from the vicinity of the College of Agriculture reserve alumina contents of 6.00 ± 0.30 per cent in the surface soil and 7.60 ± 0.22 in the subsoil. Reserve alumina in Albay soil was consistently higher than in College soil, but the difference was not as impressive as that noticeable in available aluminum of the two localities (table 2). In addition, the latter form has more direct and immediate effects on plant development. It is therefore conceivable that soils could vary in levels of toxic aluminum even if they contain the same amount of total or reserve aluminum. This consideration is probably essential to a rational view of the various and varying intensity of cadang-cadang decline among contiguous plots.

Using the bioassay technique, Velasco (1957) found that copper was generally low in the cadang-cadang areas, while zinc was equal to, if not more than that in College soil. In this study determinations of tin gave inconsistent results but indications were that Pili soil had generally higher contents than College soil.

CULTURAL EXPERIMENTS

Soil cultures

Field experiments in the coconut groves of Pili, Camarines Sur, and Nasisi, Ligao, Albay, have been started. The effects of copper sulfate, ammonium sulfate, superphosphate, potassium sulfate and lime added to the soil singly or in combination are being studied. The main criteria of results are improvement of the general appearance of the trees and the number of nuts on them. Because the reaction of the trees is rather slow, the data so far obtained do not show a clear trend and are deemed not worth reporting here.

However, the following results on experiments with short-lived plants are being presented for whatever preliminary indications they may give.

1. *The effect of copper, zinc and nitrogen.* With kamote-kamotihan (*Ipomoea triloba* L.) as the test plant, an experiment was run

to determine the effects of copper, zinc and nitrogen. Eighteen pots containing about four kilos of soil from Pili, Camarines Sur, were divided into six lots of three pots each. Lot I was left untreated and served as control; lot II was treated with 20 cc. of one per cent zinc sulfate solution per pot; lot III, with 20 cc. of one per cent copper sulfate solution; lot IV, with 2 grams of ammonium sulfate; lot V, with 2 grams of ammonium sulfate plus 20 cc. of one per cent zinc sulfate; and lot VI, with 2 grams of ammonium sulfate plus 20 cc. of one per cent of copper sulfate. The heavy application of fertilizers was designed to hasten the onset of changes in the soil (among which is the lowering of pH) which would take years if standard rates were to be applied. The pots were seeded on February 22, 1958, and treatments were made on March 7, 1958.

The plants as they appeared at five days after the treatments, are shown in figures 1 to 4. The plants with zinc sulfate alone or copper sulfate alone appeared essentially similar to the control (untreated) and hence, are not presented here. Those treated with am-

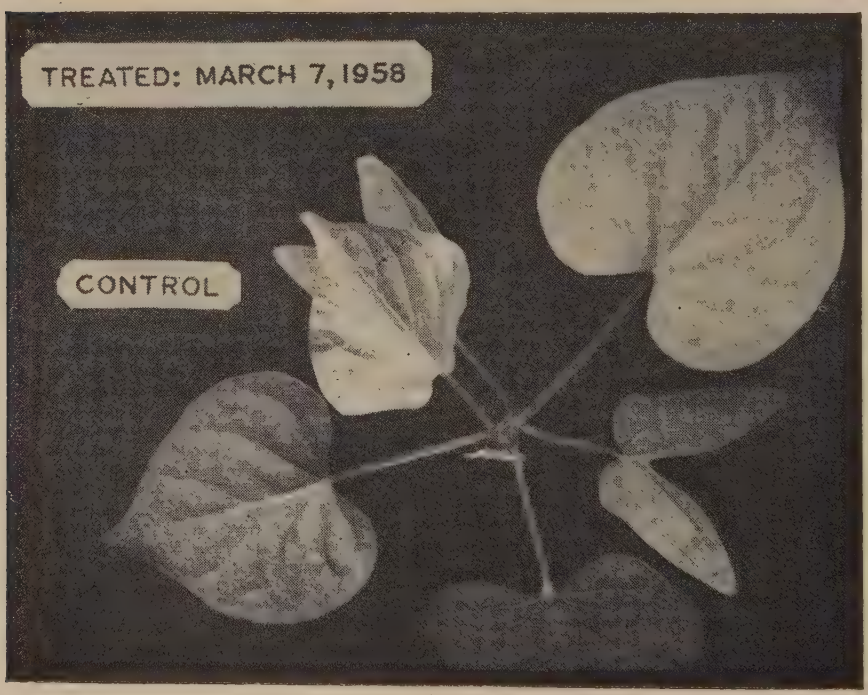

Fig. 1.—Ipomea grown in Pili soil for 18 days.

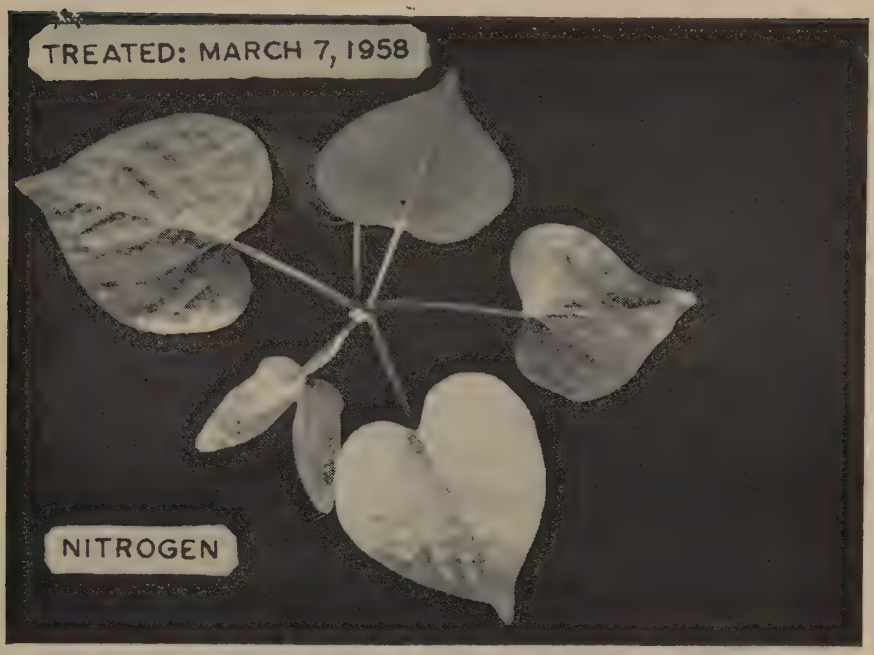

Fig. 2.—Eighteen-day-old plant, 5 days after treatment with ammonium sulfate.

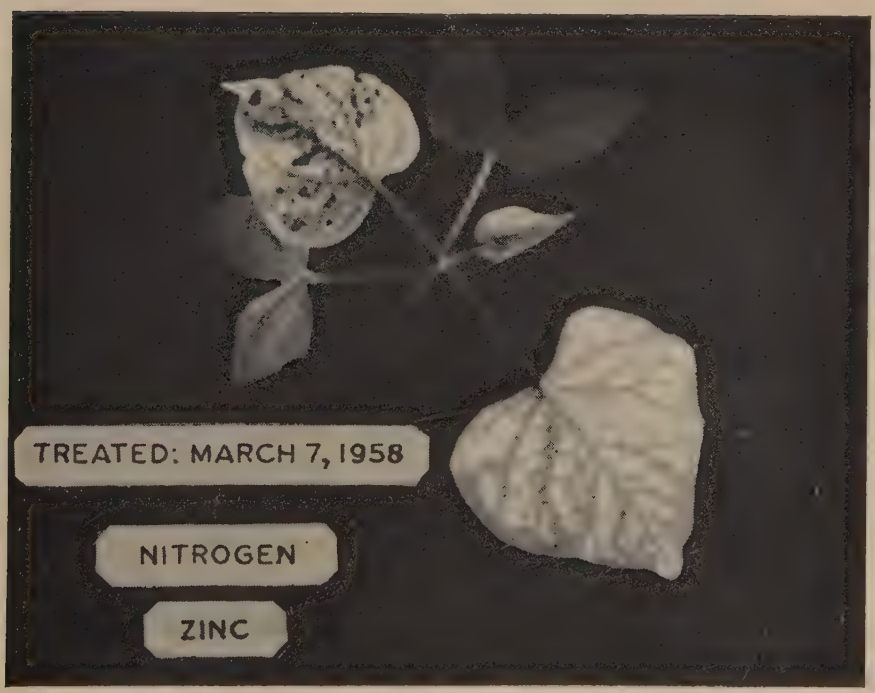

Fig. 3.—Eighteen-day-old plant, 5 days after treatment with ammonium sulfate and zinc sulfate.

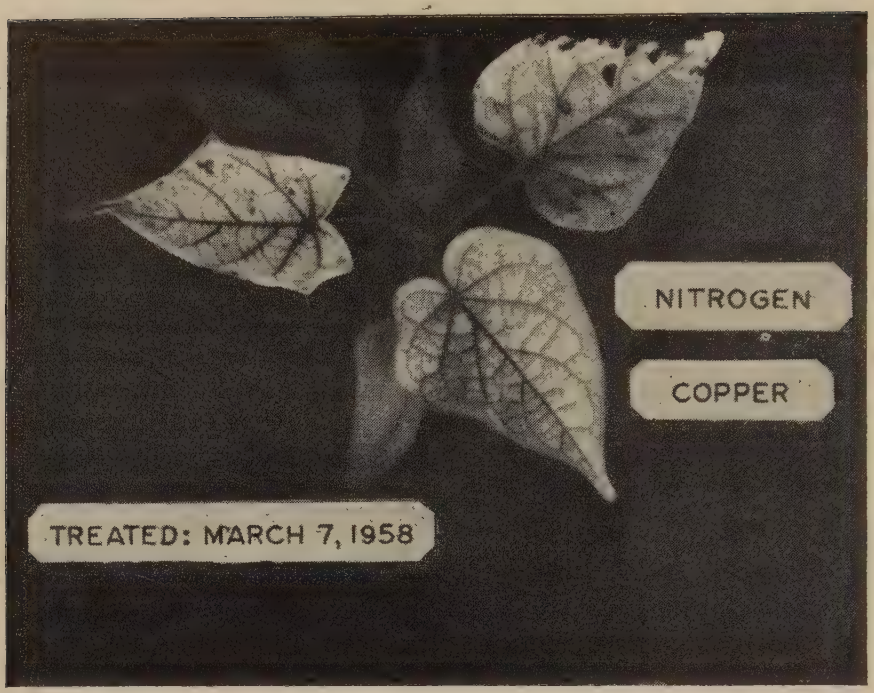

Fig. 4.—Eighteen-day-old plant, 5 days after treatment with ammonium sulfate and copper sulfate.

monium sulfate looked vigorous; their leaves tended to be rugose, as if vein-islets were out-growing the veins and veinlets. The lot that received the combination of zinc sulfate and ammonium sulfate produced stunted and highly chlorotic young leaves. They produced necrotic spots which coalesced to form blotches. The plants improved after some time but they did not grow as much as those given other treatments (table 3). Not as badly affected were those plants which received the combination of copper sulfate and ammonium sulfate.

The differences in height of the plants and dry weight of tops among lots I to IV were probably not significant. But differences between these lots on one hand and lots V and VI on the other were too great to be ignored. Since each of the materials alone did not produce the bad effects and since copper sulfate combined with ammonium sulfate was almost as bad as zinc sulfate plus ammonium sulfate, it may be held probable that the ill effects of these materials were not direct. The combinations might have released a detrimental

constituent in the soil from its innocuous binding. One plausible mechanism is that the zinc or copper cation would displace the harmful substance like aluminum from the soil colloid, and the ammonium sulfate will create conditions for the substance to be most harmful.

TABLE 3.—*Effects of applying zinc sulfate, copper sulfate and ammonium sulfate to Pili soil on the growth of Ipomoea triloba L.*[a]

LOT	TREATMENT	AVE. HEIGHT	AVE. DRY WEIGHT
		cm.	*g.*
I	No treatment	163.30	3.44
II	Zinc sulfate	158.23	2.32
III	Copper sulfate	175.56	3.11
IV	Ammonium sulfate	167.76	2.64
V	Ammonium sulfate plus zinc sulfate	6.80	0.23
VI	Ammonium sulfate plus copper sulfate	50.90	0.62

[a] The test lasted from February 22 to April 2, 1958.

Incidentally, a similarly poor growth of Ipomoea was noticed in the fields of Pili after the first rain in May. Probably much of the constituent was weathered during the hot, relatively dry months of March and April; and because there was not enough rain to leach or dilute it out, it accumulated in toxic concentration.

2. *Lime as an alleviating factor.* At this time of the study, evidence from chemical analysis was suggestive that aluminum could be a detrimental factor in the cadang-cadang problem. Thus it was thought a profitable approach to find ways of rendering it innocuous.

Other workers (Rice, 1916; Hartwell and Pember, 1918) found that fairly alkaline reaction of the soil would render the aluminum cation insoluble or largely unavailable. Since lime is among the cheapest materials that would raise the pH of the soil, liming of a series of pots corresponding to those in the previous experiment was decided. On November 26, 1958, triplicate pots were given 10 grams of ground limestone each. These pots had previously been treated with zinc sulfate, copper sulfate, ammonium sulfate and their combinations, just like in the previous experiment. They were used as a counterpart of the foregoing series in an unsuccessful attempt to determine the relative effects of diffuse light and direct sunlight on the incidence of certain abnormality in indicator plants.

On December 28, 1958, the pots were planted with 15-day-old tomato seedlings. Sixteen days after transplanting, the respective

pots were given the zinc sulfate, copper sulfate and ammonium sulfate treatments. Hence, there were two series of parallel treatments, one with lime and the other without it, and each pot was given its respective fertilizer treatment for the second time (table 4).

The pH values of the soil at 55 days after the lime treatment are given in table 4. Liming raised the pH of the unfertilized soil by 0.4 unit, that of the zinc sulfate-treated soil by 0.65, and that of the ammonium sulfate-treated soil by 1.00. Two successive treatments with zinc sulfate or copper sulfate within a space of one year lowered the pH of the soil by 0.4 unit, while a similar treatment with ammonium sulfate lowered soil pH by 1.70 units. However, in spite of this marked reduction in pH, ammonium sulfate produced no noticeable untoward effect on the tomato plants. It was rather the combination of reduced pH and the presence of zinc sulfate or copper sulfate (lots V and VI) which induced the abnormal situation resulting in poor growth of plants. This seems to strengthen the supposition that zinc and copper help release the detrimental factor of the soil from its binding and the acidic conditions brought about by ammonium sulfate, and abet the destructive action of the released constituent.

TABLE 4.—*The pH of the soil at 55 days after liming and growth of tomato plants in Pili soil under various treatments*

LOT	TREATMENT	pH OF SOIL		DRY WEIGHT IN GRAMS [a]	
		Not limed	Limed	Not limed	Limed
I	No treatment	6.15	6.55	4.2	2.6
II	Zinc sulfate	5.75	6.40	3.6	1.9
III	Copper sulfate	5.75	6.40	3.1	2.6
IV	Ammonium sulfate	4.45	5.45	12.4	11.7
V	Ammonium sulfate plus zinc sulfate	4.40	5.30	3.4	5.0
VI	Ammonium sulfate plus copper sulfate	4.20	5.50	2.16	9.6
VII	College soil	—	—	22.2	—
VIII	College soil plus 2 grams ammonium sulfate	—	—	33.2	—

[a] Harvested on February 10, 1959 or at 59 days old.

Those plants which received ammonium sulfate, especially in the limed soil produced dark-green, almost bluish green, thick leaves—in contrast with the normal green or light green color of the plants in College soil, similarly fertilized with ammonium sulfate.

In contrast with College soil, plants in Pili soil tended to produce many adventitious roots just above the ground level, and many white rootlets creeped up to the soil surface. This was noticed in all treatments, but it was more striking in the plants which received ammonium sulfate.

It will be noticed that forty-eight days were allowed to lapse between liming and the application of the fertilizers. This was done because it was feared that application of ammonium sulfate soon after liming would result in the formation of ammonium carbonate which is toxic to plants. But even with this precaution the plants in lots IV, V and VI of the limed series were retarded in growth for some time. However, in lots V and VI, the plants soon recovered and outgrew the corresponding lots of the unlimed series (table 4).

Another striking difference between the unlimed and limed series was the presence of profuse guttation in the latter. This occurred every morning after fairly cool and humid nights. (It was observed later that tomato plants treated with 2.5 ppm of aluminum as aluminum sulfate failed to guttate even in saturated atmosphere under a bell jar.)

Solution cultures

1. *Growth of tomatoes in various fractions of the soil extract.* About 500 grams of soil samples from the three localities were extracted with 500 ml. of 2 per cent HCl for 24 hours. Aliquots of the extracts were fractionated into the five groups of Noyes' scheme of qualitative chemical analysis (Noyes, 1922). Group III or the alkaline sulfide group was further subdivided into the aluminum and iron groups. The fractions were evaporated to dryness after treatment with concentrated HCl and weighed. They were then picked up in distilled water to a definite volume. Aliquots were diluted with Hoagland's solution until tomato seedlings transferred into them would no longer wilt overnight. In its final dilution the aluminum group had a concentration of 5 μg/ml.; the iron group, 5 μg/ml.; and the acid sulfide group (group II), 2.5 μg/ml. These dilutions were employed in growing tomato seedlings for eighteen days. Group I was not included in the test because there was very little of it.

Two-week-old tomato seedlings previously grown on sand were transferred to Hoagland's solution and allowed to re-establish themselves for one week. Then they were transferred to the fractions of the soil extract.

The plants grown in the alkaline earth group and the alkali group (groups IV and V) for 17 days were very healthy and indistinguishable from those grown in Hoagland's solution alone. The plants in groups II and III (acid sulfide and alkaline sulfide groups) are shown in figures 5 to 7.

In the acid sulfide group, the College sample came out worst. But since there is no cadang-cadang in the College, this may not be

Fig. 5.—Thirty-day-old tomato plants grown for 17 days in group II (acid sulfide group).

Fig. 6.—Similar to plants of fig. 5, but grown in the iron group.

Fig. 7.—Similar to plants of fig. 5, but grown in the aluminum group.

suspected as contributory to the problem. It is likely that this is a constituent which is normally unavailable and at least for the present should not be a cause for worry.

In the iron group, plants grew biggest in the College sample and least in the Pili sample. Those in the Nasisi sample assumed an intermediate position. The performance in the Pili sample was probably a reflection of its high manganese content.

The aluminum group of the three soil extracts promoted about the same amount of growth. This may be taken to mean that this group does not contain a constituent which would set apart the Bicol samples from the College sample. Whatever difference there is among the samples with respect to the aluminum group may be deduced to be one of quantity rather than composition. One striking characteristic of these plants was that they had dark-green or bluish green leaves which were thick and succulent. It will be recalled that this was the characteristic of plants in Pili soil fertilized with ammonium sulfate.

2. *The effect of aluminum on Ipomoea, tomato and mung bean.* The following experiment was run in order to find the effect on plants of various concentrations of aluminum.

On March 30, 1959, seeds of kamote-kamotihan (*Ipomoea triloba* L.) tomato and mung bean (*Phaseolus aureus* Roxb.) were germinated on tap water. At approximately one week of age they were

transferred to Hoagland's culture solution containing 2.5, 5.0, 10.0 and 20.0 ppm of aluminum as aluminum sulfate. Three plants were allowed to each treatment.

Two days after the transfer, the Ipomoea plants produced chlorotic young leaves even at the lowest concentration of aluminum. The toxic symptoms appeared on tomato six days after the transfer and on mung bean ten days after the transfer. Figures 8 to 13 show the plants at two weeks of treatment. There was gradual diminution of growth in Ipomoea with increasing concentration of aluminum (fig. 8). The roots showed definite inhibition at 20 ppm of aluminum at which concentration, the root was coral-like with short stubby branches (fig. 11, table 5).

The suppression in growth of tomato was very marked even at the lowest concentration of aluminum (2.5 ppm). The roots were correspondingly affected. In mung bean the adverse effect was marked at 5 ppm (fig. 10, table 5). Again inhibition of root development paralleled that of the shoot.

Magistad (1925) and Eisenmenger (1935) claimed that the primary effect of aluminum poisoning is in the roots.

3. *The effects of amount of iron, pH and the forms of aluminum on growth of tomatoes.* In the first of these short experiments the

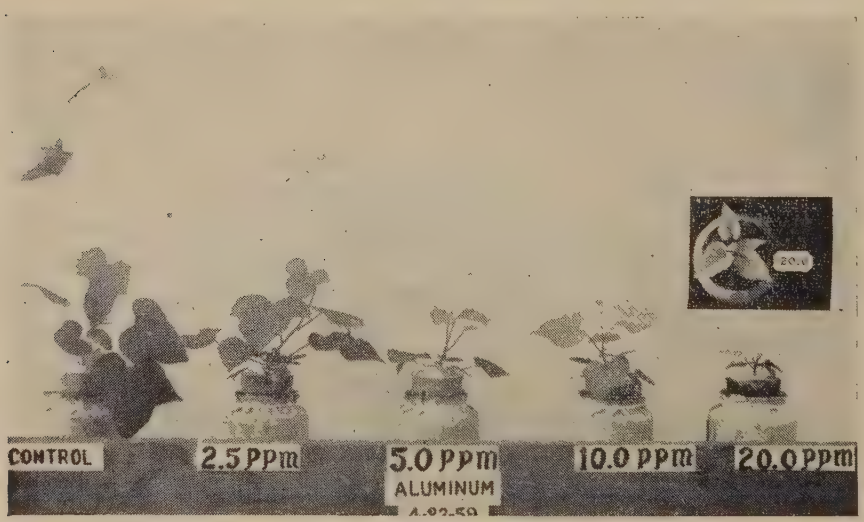

Fig. 8.—The effect of different concentrations of aluminum (ppm) on three-week-old *Ipomoea triloba*. Treatment was started at the age of one week.

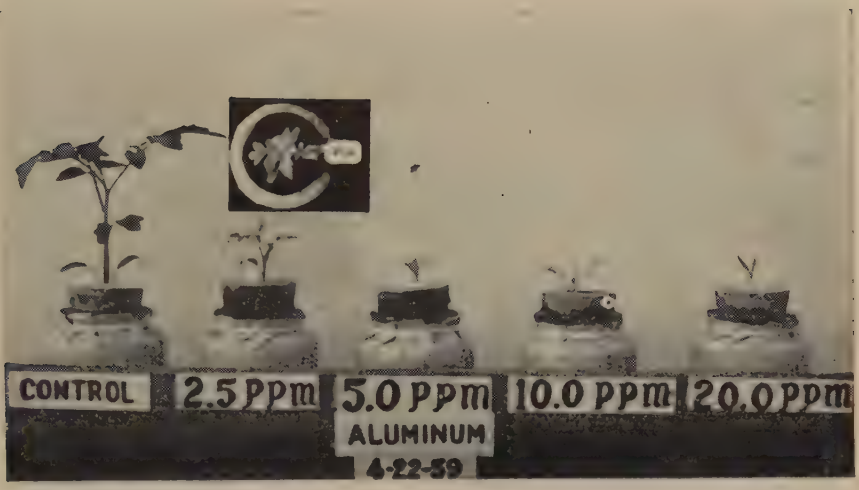

Fig. 9.—The effect of different concentrations of aluminum (ppm) on three-week-old tomatoes. Treatment was started at the age of one week.

Fig. 10.—The effect of different concentrations of aluminum (ppm) on three-week-old mungo. Treatment was started at the age of one week.

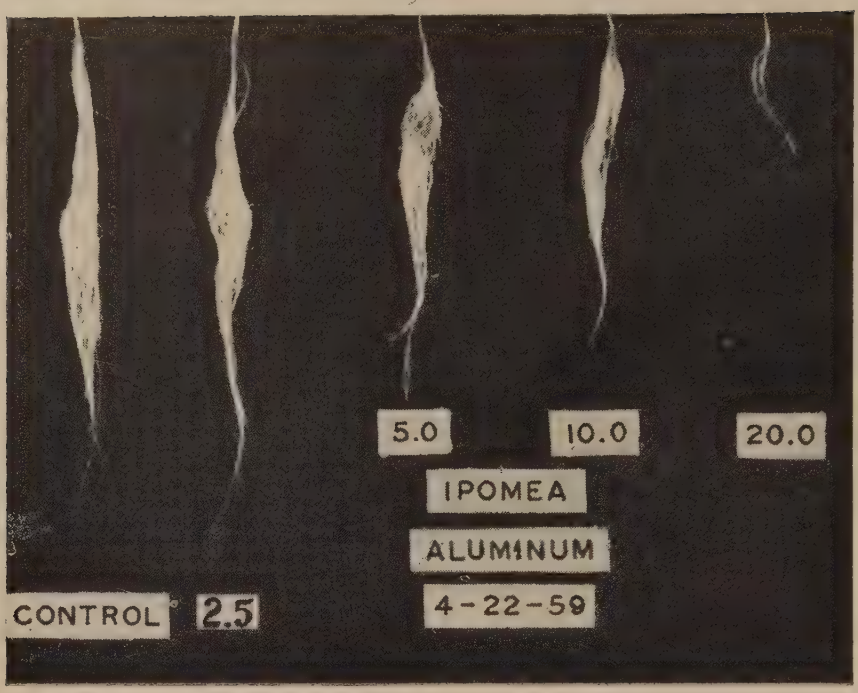

Fig. 11.—The effect of aluminum on the roots of *Ipomoea triloba* after two weeks of treatment.

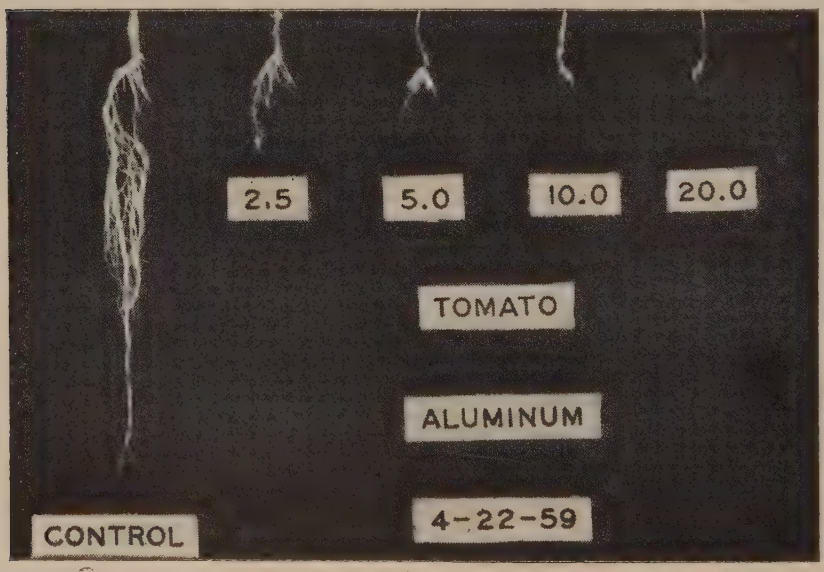

Fig. 12.—The effect of aluminum on the roots of tomatoes after two weeks of treatment.

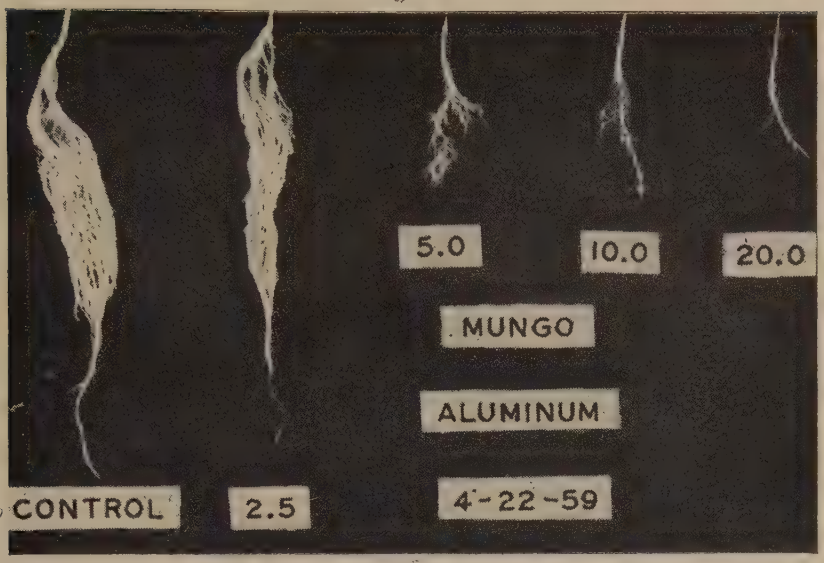

Fig. 13.—The effect of aluminum on the roots of mungo plants after two weeks of treatment.

amount of aluminum was held at 2.5 ppm and iron as ferric chloride was varied from 0, 2.5 and up to 40 ppm in a geometric progression. Chlorosis was alleviated by 40 ppm of iron, but the plants did not fare any better because the high iron concentration brought along its own toxic property.

In the second experiment, aluminum was held at 2.5 ppm while the pH was varied from 3 to 8 at intervals of approximately one pH unit.

The plants at pH 3 and 4 were stunted and chlorotic. Their roots were short, profusely branched and coral-like in appearance. Many adventitious roots were initiated on the lower part of the stem. Those at pH 5, 6, 7, and 8 had dark-green or almost bluish-green leaves, similar to plants grown in Pili soil fertilized with ammonium sulfate and those in the aluminum fraction of the soil extracts. Best growth was obtained at pH 6 and 7.

The observation seemed to warrant the hypothesis that in these various pH treatments two forms of aluminum alternatively predominate. At pH 3 and 4 the aluminum cation (Al^{+++}) was dominant and effected the chlorosis of leaves and the coralline roots. At

pH 6 to 8 the aluminate anion was preponderant. The bluish-green, thick leaves may be attributable to it.

To follow up the hypothesis, a third experiment was run to determine the toxic level of the aluminate anion. One gram of aluminum chloride was dissolved in dilute sodium hydroxide solution. The solution was evaporated on a hot plate, and at the terminal phase of evaporation, drops of 30 per cent hydrogen peroxide were added to the mass. This was calculated to convert all the aluminum into aluminate. The residue was made up to volumne with distilled water and from this stock solution, various concentrations of aluminate in Hoagland's solution were made up. Of the concentrations tried (0, 2.5, 5, 10, 20 and 40 ppm) only 40 ppm elicited abnormal symptoms on the tomato plants. They were stunted, wiry, and the young leaves were mottled yellow. The plants in 2.5 to 20 ppm were healthy and bluish green with thick leaves. The control plants had green to light green, thin herbaceous leaves.

DISCUSSION

Chemical analysis of the soil samples from the Bicol region showed that available aluminum was in relatively high concentration. However, concentration is not the only factor that determines the extent of toxicity of aluminum. Its detrimental effects are aggravated by such varied factors as low soil pH (Rice, 1916; Hartwell and Pember, 1918), low amount of phosphate (Magistad, 1925), low silica-to-alumina ratio (Mattson and Hester, 1933), low level of copper (Liebig, Vanselow and Chapman, 1942), the anions citrate, nitrate and chloride (McLean and Gilbert, 1927; Joffe and McLean, 1928), high concentration of electrolyte (Pierre, Pohlman and McIlvaine, 1932) and low amount of organic matter (Ganzha, 1941). The form of aluminum may also condition its effects. For instance, Gapon and Voshchinskaya (1941) concluded that about 90 per cent of the exchangeable aluminum in podzols is present as the oxy- aluminum cation $Al_2O_3H^+$ and that the chloride and particularly the sulfate of this ion are less injurious to wheat than are the aluminum chloride and aluminum sulfate.

The soil from Pili when treated with ammonium sulfate and zinc sulfate or ammonium sulfate and copper sulfate produced Ipomoea plants which showed symptoms of poisoning similar to that brought about by the aluminum cation. This consisted of chlorotic young leaves suggesting iron deficiency and many adventitious roots arising just above the ground level. When the soil was treated by

ammonium sulfate alone, the plants grown on it were dark green or bluish green and the leaves were noticeably thick.

When two parallel sets of soils in pots (one limed and the other unlimed) were given ammonium sulfate, zinc sulfate and copper sulfate in various treatment combinations the limed pots promoted generally improved growth of tomatoes. The differences between the limed and unlimed series were marked in pots with ammonium sulfate plus zinc sulfate and in those with ammonium sulfate plus copper sulfate.

Culture solution studies indicated that the aluminum cation was more toxic than the aluminate anion. At as low a concentration as 2.5 ppm of the cation, symptoms of toxicity were noticed on tomato plants. On the other hand, even at 40 ppm of the anion, plants were almost normal in their growth. (It is probable that the aluminate anion in this paper is related to Gapon and Voshchinskaya's oxyaluminum cation. No attempt was made to elucidate this point.)

From this information, the writers are tempted to postulate that the high level of available aluminum and the low level of copper (Velasco, 1957) are two disturbing factors in Bicol soils which may cause the cadang-cadang of coconut. Toxic levels of aluminum may bring about impairment of the water-absorbing functions of the roots. This is reflected in the successive reduction of the leaves, as if the plant is undergoing a series of self-pruning in order to adjust itself to a diminishing water supply. This is capped by the loss of the entire crown. Deficiency of copper may cause heavy premature fall of nuts, and in severe cases, inhibition of the fruit bunches.

One of the important functions of an hypothesis is to explain or integrate the many bits of information on the subject. The following discussions are an attempt to relate the observations on cadang-cadang with the postulated aluminum-copper hypothesis.

1. *Clean culture and periodic plowing under of the ground cover aggravate the disease.* The organic matter which is plowed under decomposes under partially anaerobic conditions. Some of the intermediate products of anaerobic decomposition are organic acids which may render the soil acidic. The reducing conditions may also encourage more of the aluminum cation to form.

Another source of difficulty with this practice is that periodic plowing exposes more of the soil to weathering and may render available a greater fraction of soil aluminum.

2. *Application of fertilizers aggravates it.* Discussion will be limited to ammonium sulfate, superphosphate, and potassium chloride or sulfate because they are most commonly applied in fields.

Ammonium sulfate application elicits in about two months the production of dark-green and apparently very healthy leaves. However, this is followed by an abrupt decline and in two or three years after the application, the coconut trees may become worse than trees which have not been fertilized at all. This may be explained by the fact that ammonium sulfate has an acidic residual effect on the soil. The high acidity would render available more of the aluminum and aggravate its toxic effects.

Some cases of cadang-cadang decline are partly traceable to lack of nitrogen in the soil, so that despite the incidental effects of nitrogenous fertilizers in rendering available and augmenting the toxicity of aluminum, farmers may still need to apply nitrogen. It seems therefore worthwhile to find the best form of nitrogen to apply and the method of applying it effectively. New methods of application like foliar sprays with urea may deserve to be explored.

The effects of superphosphate have not been closely studied. Some fieldmen in the Bicol region claim that superphosphate has not alleviated the condition of trees suffering from cadang-cadang. But this claim is based on casual observation which may extend not later than six months after the application of the fertilizer. It is felt that a thorough and systematic study is urgently needed. Based on indirect information, it may be expected that regular application of superphosphate, or better still phosphate rock, would alleviate the condition of coconut trees in the Bicol region. The phosphate ion may be expected to drag down the available aluminum and render it innocuous. Phosphate rock may buffer the soil at a relatively high pH, beyond the range at which aluminum can be most detrimental.

Potassium chloride or potassium sulfate as it relates to cadang-cadang has not been thoroughly studied. Again, interest in it has not been sustained long enough to obtain unequivocal results. There is the possibility that these salts would increase the electrolyte in the soil and make available more of the aluminum (Pierre, Pohlman and McIlvaine, 1932). If this can somehow be counterbalanced by other soil management practices like manuring, then much could be gained by fertilizing with potassium. Coconut has been noted to be a heavy consumer of potassium.

3. *Cadang-cadang is serious in low places.* Low places or flatlands are badly affected by the disease because all the soluble aluminum in the higher places tend to accumulate in them. Besides that, these areas are porrly drained, hence, are partially anaerobic. This condition promotes the conversion of aluminates into the aluminum cation. Groves which are on rolling land should fare better provided that the slope is not so steep that practically no more top soil remains.

4. *The disease is predominant among tall trees.* Tall trees have to exert more effort to mobilize water from the roots to the leaves in the process of transpiration. When there is a disruption in the water economy of the plant the tall trees suffer more than the shorter ones. It was demonstrated in tomatoes that guttation of water (an indication of root pressure) is adversely affected by aluminum. Whether or not root pressure is involved in the ascent of water to the top of tall trees is a moot point, but the idea here conveyed is that water economy in the plant is disrupted upon treatment with aluminum. Diminution of root pressure is probably just one manifestation of that disruption.

5. *Incidence is prevalent during the few months following a strong typhoon.* This may be explained by the observation that roots of some plant species exposed to high levels of aluminum tend to develop a relatively impervious layer (von Faber, 1925) so that little or no aluminum could diffuse into the stem and leaves. During typhoons, however, this defensive system is destroyed because the violent shaking of the plant causes the rupture of many roots. Toxic amounts of aluminum could then enter the plant through the cuts and bring about symptoms of aluminum poisoning which in coconut is diagnosed as cadang-cadang.

6. *Starch is low in the leaves and trunk of diseased trees.* Some workers claim that starch is low in leaves of cadang-cadang-affected trees, but as far as the writers are aware there is no published account of this. Assuming that the claim were true, low starch content is only to be expected as an offshoot of aluminum poisoning. Fluri (1906) found that cells become less subject to plasmolysis after treatment with aluminum and that starch disappeared from the cells of certain water plants. He supposed that enzymatic hydrolysis was increased, photosynthesis inhibited and permeability increased, allowing loss of sugar.

Aspiras[2] noted more simple sugars (reducing and non-reducing) in the "cabbage," the growing point and the trunk of healthy trees than those of cadang-cadang-affected trees.

Post script. The writers are aware that the reaction of short-lived plants like Ipomoea and tomato to various forms of aluminum under modified laboratory conditions can not be held to apply strictly to field-grown coconuts. The choice of the plant "guinea pigs" was dictated more by expediency and by the casual observation that these plants were not growing normally in the fields of Bicol. But the approach commends itself on these grounds:

Now that suspicion lies heavily on the soil constituents, field experiments on coconut would not have to fumble along many uncertain lines. Extensive and thorough study of the soil in the Bicol region, with emphasis on aluminum and copper, may now be initiated with some measure of confidence. Effects on coconut of excess aluminum and/or deficient copper may be studied in detail under controlled conditions. Field tests designed to correct the excess of aluminum in the soil in which coconuts are growing, may yield important results, useful in the management of coconut groves in the Bicol region.

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RADIATION GENETIC STUDIES ON RICE (*O. SATIVA*)[1] I. OBSERVATIONS AMONG THE X_1 PLANTS

FILOMENA F. CAMPOS, JOSÉ M. CAPINPIN
and LUCILA G. ESPIRITU

Results obtained from subjecting dormant unhulled and hulled rice grains to various levels of X-rays showed that germination was relatively less affected among the unhulled than the hulled lots. X-ray levels beyond 10 kr resulted in the inhibition of the growth of the radicle of all treated seedlings irrespective of hull treatment. Rate of growth of the phenotypically normal X-rayed seedlings indicated an inverse relation to X-ray dosage. Highest frequency of induced mutants was recorded at 10 kr. Majority of them were chlorophyll-deficient seedlings, all of which did not survive beyond transplanting. No significant difference was obtained among the treated lots with regard to number of tillers, length and straw weight.

A profusely tillering mutant was isolated from the hulled lot subjected to 2 kr. The number of productive tillers produced by this mutant was three times that of the control. Grains of said plant exhibited a characteristic segregation of hull and endosperm color.

Since the discovery of the mutagenic effects of X-rays, several researches have been conducted in plants with the aim of inducing viable mutants which could serve as possible genetic stocks for breeding. Although it has been established that most X-ray-induced mutants are mostly undesirable (Stadler, 1930; Sax, 1955), occasional 'genetic off-types' which have tremendous possibilities for breeding do arise.

This investigation was conducted to determine the critical dosage to X-radiation of Milfor 6 rice variety, to observe the morphogenetic effects of various X-ray dosages on rice seeds, and to analyze and evaluate useful and viable mutants arising from X-rayed rice seeds.

MATERIALS AND METHODS

Unhulled and hulled dormant grains of Milfor 6(2)[2] were subjected to X-rays at dosage levels of 2 kr (kilo roentgen), 5 kr, 10 kr,

[1] Central Experiment Station Contribution No. 2006. Rice and Corn Research Project No. 15.

[2] Furnished by the Division of Plant Breeding, Department of Agronomy, U. P. College of Agriculture.

20 kr, and 50 kr. X-rays were applied without a filter by a General Electric Maximar 100 operated at 225 roentgen per minute, 100 kv (kilo voltage), 50 ma (milliampere) and at a target distance of 15 centimeters.[3]

One hundred seeds for each dosage lot were irradiated and immediately germinated on moist filter paper in an improvised germinator having a temperature ranging from 35-38°C. Data on percentage germination were obtained over a week's period after which the seedlings were planted in half-drum containers. Preliminary observations on the types and frequency of induced mutants from these seedlings were made prior to transplanting in the field. Mutants obtained were isolated and individually observed.

Thirty phenotypically normal plant samples per lot were grown and studied to maturity.

RESULTS AND DISCUSSION

Table 1 shows the effect of X-rays on the germination of the unhulled and hulled grains. Irrespective of hull treatment[4] the control lots did not vary significantly from the treated lot up to 10 kr. However, comparisons between hull treatments showed that the unhulled lots were relatively less affected by X-rays than the hulled lots except for the hulled lot treated at 2 kr.

TABLE 1.—*Percentage germination of rice seeds subjected to varying X-ray dosages* [a]

DOSAGE	UNHULLED GRAINS	HULLED GRAINS
Control	100	100
2 kr	100	90
5 kr	95	90
10 kr	90	85
20 kr	80	80
30 kr	45	35

[a] Average of 2 trials.

During the first days of the seedling's growth the radicle and plumule of the treated seedlings did not show any change in external appearance. However, after a week's period several plants from

[3] X-rayed by the X-ray Division, Philippine General Hospital, Manila.
[4] Hull treatment refers to unhulled and hulled lots.

both unhulled and hulled lots irradiated at level beyond 10 kr exhibited a definite stunting of the radicle with the end results of stubby roots with no formation of lateral roots (fig. 1). These plants did not survive beyond transplanting. Ohta *et al.* (1957) observed similar effect on rice seeds exposed to Co^{60} at dosage levels beyond 4 kr. This finding also corroborates the findings of Smith and Kersten (1914) on X-rayed seeds of *Vicia faba.*

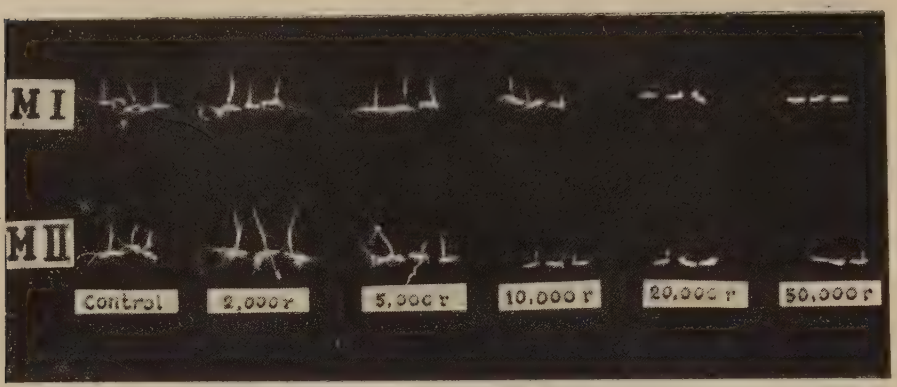


Fig. 1.—Type and rate of growth of rice seeds treated at different levels of X-rays. (MI is unhulled, MII, hulled).

Although several workers (Koernicke, 1904, 1915; Johnson, 1936; Shull and Mitchell, 1933; Wort, 1941) and a number of Russian reports attested to the stimulatory effect of X-rays on plant growth, results of this experiment proved otherwise. These might have been due to a higher dose employed.

Rate of growth of the seemingly normal seedling indicated an inverse relation to X-ray dosage (table 2). Regardless of dosage

TABLE 2.—*Rate of growth in centimeters of rice plants from seeds subjected to X-rays*

DOSAGE	UNHULLED GRAINS			HULLED GRAINS		
	4 days	10 days	30 days	4 days	10 days	30 days
Control	0.68	3.62	52.69	0.91	4.73	55.09
2 kr	0.78	2.93	57.25	1.11	4.88	50.29
5 kr	0.62	2.75	49.64	0.91	3.69	40.36
10 kr	0.63	2.52	44.71	0.89	3.04	24.90
20 kr	0.36	1.98	18.00	0.51	2.66	16.00
50 kr	0.43	1.54	16.00	0.43	0.95	11.00

all the unhulled lots exhibited a more marked growth rate than the hulled lots except that treated at 2 kr.

Except for one profusely tillering plant isolated from the hulled lot treated at 2 kr no effect of X-ray treatment was reflected on the number of productive tillers produced in both the unhulled and hulled lots.

Data obtained from panicle length showed that only the hulled lot treated at 50 kr was longer than the unhulled lot exposed to the same X-ray dosage. Percentage of filled grains was affected inversely by X-ray dosage; higher dosage resulted in decrease in percentage filled grain. However, weight per 100 filled grains revealed that significant differences exist among 2 kr, 5 kr, 20 kr lots irrespective of hull treatment. Except for the lot treated at 2 kr the other treated lots did not differ significantly in mean value with regard to straw weight.

Several X_1 mutants were isolated from the treated lots. The highest frequency of induced mutants was recorded from the hulled seeds irradiated at 10 kr. Majority of these mutants were chlorophyll-deficient plants which did not survive beyond the transplanting stage (fig. 2).

Fig. 2.—Chlorophyll-deficient seedling isolated from irradiated seeds.

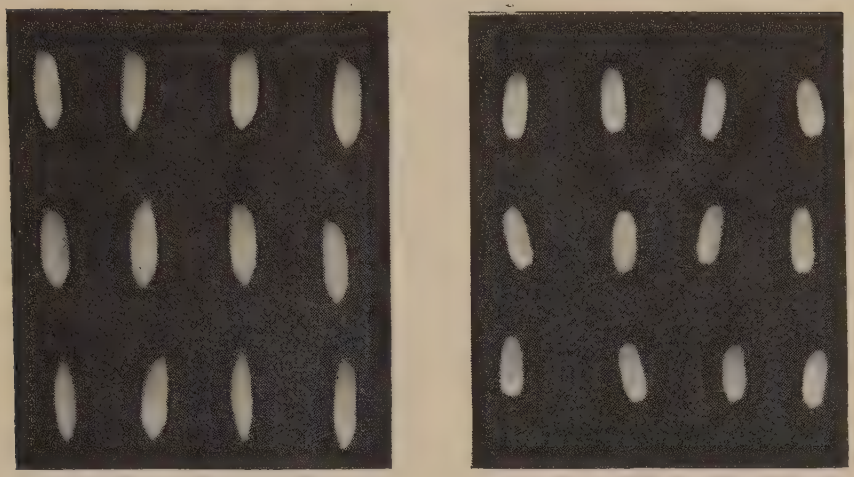
TABLE 3.—*Average plant height, number of tillers, per cent of productive tillers (% PT), panicle length in cm., weight in grams per 100 grains (G/100), per cent filled grain (% FG) and weight of straw in grams*

	UNHULLED							HULLED						
DOSAGE	Height Plant	Number of Tillers	% PT	Panicle Length	G/100	% FG	Weight of Straw	Plant Height	Number of Tillers	% PT	Panicle Length	G/100	% FG	Weight of Straw
				cm.			*g.*				*cm.*			*g.*
Control	123.00	15.17	70.35	29.02	3.39	66.07	57.05	116.86	14.70	73.53	29.63	3.44	51.50	63.04
2 kr	126.16	14.56	73.76	28.90	3.49	73.35	63.97	120.10	19.20	52.63	29.50	3.43	57.50	64.89
5 kr	124.53	15.53	66.61	28.85	3.34	72.03	54.89	118.96	13.17	69.48	29.49	3.33	65.28	46.55
10 kr	117.20	14.03	67.49	28.57	3.40	66.55	57.05	119.10	11.03	71.72	29.10	3.30	54.34	52.82
20 kr	107.90	18.54	20.45	28.09	3.38	13.00	49.68	114.07	13.25	60.17	28.23	3.48	53.88	51.44
50 kr	113.75	20.83	30.00	27.00	3.35	38.06	54.08	113.85	11.08	48.62	27.27	3.54	29.36	45.52

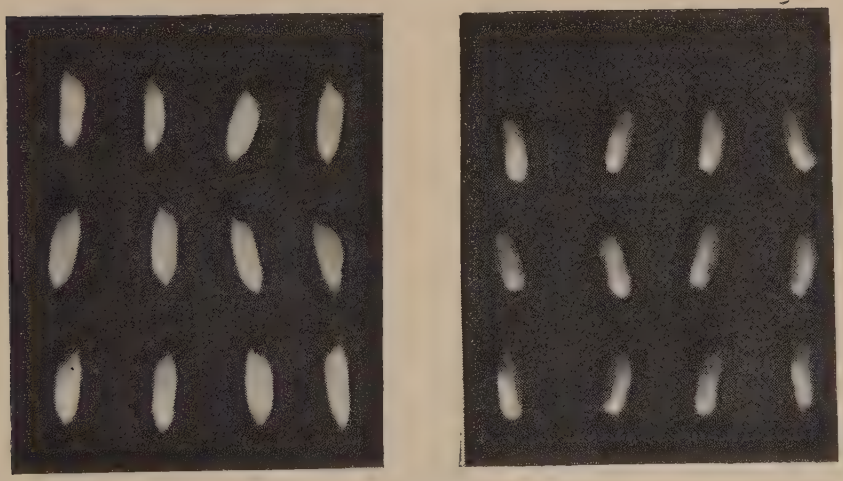
A rather promising mutant was isolated from the hulled lot treated at 2 kr. The plant from germination to almost one month old exhibited a dwarfish, bushy-type of growth, light green, with relatively narrow leaves and produced an unusually profuse number of tillers (fig. 3). Upon maturity the growth of the mutant was accelerated and at harvest time it exceeded the control. The number of productive tillers produced by this mutant was definitely three times that of the control (fig. 4). Grains produced exhibited a characteristic segregation of hull and endosperm color. Yellow hull was correlated with white endosperm (17.10 per cent) while dull brownish yellow hull with red endosperm (82.5 per cent) (fig. 5). Whether this is an effect of X-rays or just a delayed segregation from hybrid ancestry of the treated material, is a subject for further study.

Fig. 3.—Profusely tillering mutant (MIIZ) isolated from hulled lot treated at 2 kr. (1 month old).

Fig. 4.—MIIZ mutant at maturity showing number of productive tillers. MIIC—Control; MIIZn—phenotypically normal X-rayed plant; MIIZ—profusely tillering mutant.



(a)



(b)

Fig. 5.—Unhulled and hulled grains of mutant MIIZ exhibiting distinct segregation of hull and endosperm color; (a) yellow hull with white endosperm; (b) dull brownish-yellow hull with red endosperm.

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WHITE FLINT CORN IN POULTRY RATIONS[1]

E. C. Coligado, F. M. Fronda, S. C. Agpalza, and B. P. Aqui, Jr.

Three experiments were conducted to find out the feeding value of white flint corn in rations for growing chicks and for layers. It was observed that in rations supplemented with five per cent ipil-ipil (*Leucaena glauca* Linn.) leaf meal, white flint corn gave approximately the same results as yellow flint corn when fed to chicks, pullets, and layers. When not supplemented with ipil-ipil leaf meal, a good source of carotene, white flint corn fed for long periods effected reduced vitality which is due probably to lack of vitamin A.

Corn (*Zea mays* Linn.) is one of the most important grain crops raised in the Philippines. Unlike the yellow flint corn, the white flint variety is not popularly used as an ingredient in poultry rations. This may be due to claims that corn is not a good feed for animals. To find out the influence of white corn in rations for poultry, a series of experiments were conducted in the Department of Animal Husbandry, College of Agriculture, University of the Philippines, from January 1, 1955, to June 16, 1956.

REVIEW OF LITERATURE

The results of experiments by Peterson, Hughes, and Payne (1939) showed that yellow corn is high in vitamin A while white corn is not. The New Jersey Station Report in 1930 stated that yellow dent corn was found to be a more potent source of vitamin A than the white-capped dent variety. The chicks on a vitamin A–deficient ration failed to develop yellow pigmentation of the beaks and shanks. Milne (1954) reported that yellow corn is an excellent source of vitamin A. He further stated that laying hens which were fed with a level as high as 70 per cent of yellow corn in an all-mash laying ration gave good production, but the addition of manganese sulfate was necessary to compensate for low manganese content.

In his depletion experiments concerning the relation between carotene and vitamin A, Moore (1951) concluded that carotene is

[1] Central Experiment Station Contribution No. 2007. Experiments 1 and 2 were based on theses presented for graduation by S. C. Agpalza and B. P. Aqui, Jr., respectively, with the degree of Bachelor of Science in Agriculture, March, 1956. Prepared in the Department of Animal Husbandry under the direction of Research Professor F. M. Fronda and Mr. E. C. Coligado.

presumably the source of vitamin A and that pigmentation is possibly due to carotene. Drummond, Ahmad, and Morton (1931) likewise showed that vitamin A is due entirely to carotene. Spectographic tests revealed the presence of no other pigment than carotene as a source of vitamin A. They further concluded that the destruction of carotene resulted in the loss of growth-promoting properties of the substance.

Palmer and Kempster (1951), on the contrary, stated that xanthophyll and not carotene is used by the fowl for pigmentation of the beaks and shanks.

Yellow corn contains fat-soluble substances while white corn does not. This was observed by Steenbock and Boutwell (1920) in a comparative study on the nutritive value of white and yellow corn.

Morrison (1954) stated that white corn is an excellent feed for poultry if vitamin A supplement is added to the rations.

MATERIALS AND METHODS

One hundred eighty day-old chicks and sixty pullets of the Single-Combed White Leghorn breed raised from the poultry flock of the College were used in the experiments. White flint corn was taken from the Department of Agronomy. The ipil-ipil leaf meal was prepared from plants growing in the vicinity of the College and the other components of the rations were obtained from the general purchases of the Department of Animal Husbandry.

Experiment 1

White flint corn in rations for chicks. A total of 180 Single-Combed White Leghorn day-old chicks were used in three 12-week trials. The chicks were divided at random into two lots of 30 chicks to a lot. All the trials were conducted at approximately the same time.

In all the trials, the chicks of both lots were raised in electrically-heated battery brooders during the first four weeks, and then transferred to outdoor rearing cages until the twelfth week. The two lots were placed side by side throughout the experiment.

Except for the kind of corn, the same all-mash brooding ration was used in both lots. It contained the following ingredients, all parts by weight:

Rice bran	55.0
Ground corn	20.0
Soybean oil meal	11.5
Copra meal	10.0
Fish meal	7.5

Ipil-ipil leaf meal, at the rate of five per cent; Aurofac, 0.5 per cent; and salt, 0.5 per cent were added to the ration in both, lots I and II. The ration containing yellow flint corn was given to lot I and the white flint corn ration to lot II, the experimental lot.

The initial, weekly and final weights were noted. The amount of feed consumed and the number of birds that died in each lot were also recorded.

Experiment 2

White flint corn in rations for egg production. Sixty Single-Combed White Leghorn pullets of almost the same age were divided at random into two lots of 30 pullets to a lot and kept in individual laying cages. Lot I was designated as control and was given yellow flint corn in the ration; lot II, the experimental lot, was given white flint corn. Except for the kind of corn used, the same mash mixture was given to the birds of both lots. The proportion of the ingredients in each ration, all parts by weight was as follows:

Ground corn	50
Rice bran	20
Copra meal	10
Fish meal	20

Ipil-ipil leaf meal at the rate of five per cent; ground oyster shell, five per cent; and salt, one per cent were added to the mixture.

The monthly weights of the birds and daily egg production of each were recorded. All eggs laid during the first ten days of every month were taken as samples and weighed on the lot basis. The monthly feed consumption of each lot as well as mortality rate and its causes were noted.

Experiment 3

White flint corn in rations for layers on extended white flint corn rations. All the female chicks produced in experiment 1 were raised to maturity. During the rearing period, they were fed the

same kinds of corn in the rations as in the previous experiment. After reaching sexual maturity, thirty pullets from each lot were selected and placed in laying houses with adjoining range pastures. Except for the kind of corn used, the same composition of feed was given to both lots. The laying ration used was exactly similar to the ration in experiment 2 except for the exclusion of ipil-ipil leaf meal assuming that the birds would get sufficient green feeds from the pasture to supply the necessary vitamin A requirements. The pullets were observed for 365 days. Daily records of egg production, total feed consumption, and mortality rate were noted. Size of eggs was determined by weighing the daily egg production.

RESULTS

Experiment 1

Rate of growth. At 12 weeks of age, the yellow corn-fed birds had an average weight of 867.2 grams and the white corn-fed birds weighed 885.3 grams. Weekly average weights are presented in table 1. Statistical analysis showed that the difference between lots was insignificant.

Feed consumption and economy of production. The amount of feed consumed by the birds during the entire 12-week period did not vary much between the two lots. The birds in lot 1, which were fed with yellow corn, needed an average of 4.87 kilograms and those in lot II, which were given the white corn, needed 4.66 kilograms of feed to produce a kilogram gain in weight. Assuming that the weanling produced would command a price of ₱2.00 a kilogram and that one kilogram of the yellow flint corn ration cost ₱0.23 while a kilogram of the white flint corn ration cost ₱0.22, lot I gave a return of ₱66.58 and lot II, ₱76.53 or a difference of ₱9.95 more for the white corn lot for the whole experiment.

Mortality. Of the 90 chicks used in each lot, seven birds, or 7.8 per cent, died in lot I and only three birds or 3.3 per cent, died in lot II. These mortality rates in both lots are well within the normal range. Although there is a difference of almost 50 per cent in favor of the white corn lot, it is difficult to attribute its cause to the kind of corn used.

Experiment 2

Egg production and size of eggs. Table 2 shows important data obtained in experiment 2. In a period of 365 days, exactly the same

average egg production was obtained. An average production of 50.9 per cent or 186 eggs in lots I and II were obtained.

The annual mean weight of the eggs produced in lot I was 54.6 grams and in lot II, 54.3 grams. White corn in a ration for layers did not affect the size of eggs produced.

Feed consumption and amount of feed needed to produce a dozen eggs. There was practically no difference in the amount of feed consumed by each bird in the two lots. The average amount of feed consumed by a bird in one year was 36.5 kilograms in lot I, and 36.9 kilograms in lot II. To produce a dozen eggs, lot I required 2.38 kilograms and lot II, 2.39 kilograms of feed costing ₱0.61 and ₱0.59, respectively.

Mortality. Of the thirty pullets in each lot, four died in lot I, and six in lot II. The slightly higher number of deaths of birds in lot II compared to lot I are attributed not to the use of white corn in the ration, but to broken egg inside the uterus, strangulation between the cages, and leucosis complex.

Experiment 3

Egg production and size of eggs. The average egg prdouction in the yellow corn-fed lot was 136 eggs for each pullet; or 37.2 per cent, while that in the white corn-fed lot was 125 eggs or 34.2 per cent, a difference of only 11 eggs. The average weight of the eggs produced in lot I, 51.9 grams, was slightly heavier than that in lot II, 50.3 grams. The birds fed with white corn in the ration produced 11 eggs less and slightly smaller ones than the yellow corn-fed birds. If these differences in favor of the lot I pullets could be attributed to the kind of corn used in the rations, it is possible that the birds in the white corn lot were not able to get sufficient vitamin A from the pasture.

Feed consumption and amount of feed to produce a dozen eggs. During the year, the birds in lot I consumed an average of 30 kilograms of feed, while those in lot II, 29 kilograms. The slightly lower feed consumption of the birds in lot II as compared to those in lot I may probably be due to the slightly lower egg production in lot II. To produce a dozen eggs, lot I needed 2.71 kilograms of feed costing ₱0.68 and lot II, 2.76 kilograms costing ₱0.66. It cost several centavos more to produce a dozen eggs in both lots in this experiment than those in experiment 2. This could have been due to

a much lower number of eggs produced in both lots in the latter experiment.

Assuming that 60 per cent of the total cost of production is due to feed, and the cost of eggs is ₱0.12 each, lot I gave a net gain of ₱82.43 while lot II, ₱66.40. The returns above feed cost was ₱213.20 for lot I and ₱154.08 for lot II.

Mortality. Of the 30 pullets assigned to each lot, 15 or 50 per cent died in lot I and 20 or 66 per cent died in lot II. Both lots exhibited a high mortality rate caused by nutritional roup especially in lot II. This might have been due to the fact that the birds in this lot were not able to get sufficient vitamin A from the pasture. The causes of the other deaths were broken eggs inside the uterus and intestinal parasitic infestations.

DISCUSSION

In these experiments, white flint corn was compared to yellow flint corn in rations for poultry. It was fed to chicks to find its effects on growth and to layers to find its effects on egg production.

White corn for chicks. The growth of chicks was not adversely affected by feeding white corn in the ration. At twelve weeks of age, the average weight of chicks in the white corn lot was 18.1 grams heavier than the chicks fed with yellow corn in the ration. This difference was found to be insignificant. These growth results therefore indicate that white corn has the same effect as yellow corn for chicks especially if five per cent ipil-ipil leaf meal is added to the ration to furnish adequate vitamin A. Quisenberry (1947) found that ipil-ipil is an excellent source of carotene, being almost twice as high in carotene as alfalfa leaf meal. Ylagan and Ynalvez (1956) observed that the carotene of ipil-ipil dried under the sun was 9.733 micrograms per 100 grams.

Slightly less white corn ration was needed to produce a kilogram gain in weight. The white corn ration being cheaper and effecting good growth on chicks proved as profitable as, if not more profitable than, yellow corn in this experiment.

The chicks fed with white corn in the ration had a much lower death rate than that of the control.

White corn for layers. When white corn was tried on layers, the results clearly showed that it is as good as yellow corn especial-

ly if five per cent ipil-ipil leaf meal is added to the ration. However, when white corn was fed to layers in a ration without ipil-ipil leaf meal but kept in a range house, the number and size of eggs produced during the year was lower and smaller compared to the yellow corn-fed pullets.

Mortality of pullets was higher in the white corn than in the yellow corn ration when ipil-ipil leaf meal was excluded from their diet. In experiment 2, wherein ipil-ipil leaf meal was added in the two rations, the mortality rate was almost identical.

In many instances yellow corn came out as a better feed than white corn when no ipil-ipil leaf meal was added to the ration. However, figures showed that either kind of corn can be a profitable feed for layers. It is probable that better production would have resulted if ipil-ipil leaf meal was added in the rations in experiment 3 as was done in experiment 2.

These foregoing results show that if white corn is used in rations for poultry, it may be advisable that an adequate supply of vitamin A must be assured in their diet so that the results may not be influenced adversely.

TABLE 1.—*Average initial and weekly weights in grams of Single-Combed White Leghorn chicks as influenced by white corn in the ration*

WEEK	LOT I (YELLOW FLINT CORN)				LOT II (WHITE FLINT CORN)			
	Trial				Trial			
	1	2	3	Average	1	2	3	Average
Initial	39.1	36.6	36.3	37.3	36.6	37.7	36.7	37.0
1	55.0	63.3	53.3	57.2	55.6	70.0	46.7	57.6
2	82.8	86.7	76.7	82.0	85.7	90.0	73.3	83.0
3	113.8	131.0	123.3	122.7	114.3	134.5	123.3	124.0
4	165.5	203.4	173.3	180.8	146.4	227.6	183.3	185.8
5	251.7	279.3	240.0	257.0	242.9	272.4	263.3	259.5
6	337.9	362.1	330.0	343.3	321.4	382.8	360.0	354.7
7	396.6	489.7	446.7	444.3	417.9	506.9	456.7	460.1
8	558.6	562.1	536.7	552.5	553.6	596.6	556.7	568.9
9	682.8	679.3	572.4	644.8	667.9	693.1	633.3	664.8
10	793.1	768.9	593.1	718.4	782.1	782.8	663.3	742.7
11	893.1	789.7	696.3	793.0	882.1	806.9	743.3	810.8
12	955.2	810.3	836.0	867.2	975.0	827.6	853.3	885.3

TABLE 2.—*The effect of white corn in a ration for Single-Combed White Leghorn layers*

ITEM	EXPERIMENT 2		EXPERIMENT 3	
	Lot I	Lot II	Lot I	Lot II
Average weight of the birds at the start, kgm.	1.29	1.30	1.32	1.21
Average weight of the birds at the end, kgm.	1.72	1.72	1.70	1.64
Total number of eggs produced	5410.00	5230.00	3285.00	2380.00
Mean weight of eggs, grams	54.61	54.25	51.90	50.30
Total value of eggs produced, pesos [a]	649.20	627.60	394.20	285.60
Egg production per bird, per cent	50.90	50.90	37.20	34.20
Number of eggs produced to a bird	186.00	186.00	136.00	125.00
Total amount of feed consumed, kgm.	1072.00	1041.80	724.00	548.00
Total cost of feed consumed, pesos [b]	276.58	257.32	191.00	131.52
Average feed consumption per bird per year, kgm.	36.50	36.87	30.00	29.00
Cost of feed consumed per bird, pesos	9.22	8.58	7.74	7.16
Feed needed to produce a dozen eggs, kgm.	2.38	2.39	2.71	2.76
Cost of feed needed to produce a dozen eggs, pesos	0.61	0.59	0.68	0.66
Returns above feed cost, pesos	372.62	370.28	213.20	154.08
Number of birds that died	4.00	6.00	15.00	20.00
Mortality, per cent	13.33	20.00	50.00	66.00

[a] Based on ₱1.44 a dozen.
[b] Cost of a kilogram feed was ₱0.258 for lot I, and ₱0.247 for lot II.

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PERFORMANCE OF CHICKS FED RATIONS SUPPLEMENTED WITH VARYING LEVELS OF AUROFAC [1]

G. O. Ranit, R. B. Puyaoan and R. V. Alicbusan

The highest average weight and most efficient feed utilization were obtained with S. C. White Leghorn and New Hampshire chicks fed a practical-type ration and supplemented with 2.0 per cent Aurofac. At 4.0 per cent level, there was a marked decrease in average weight. Mortality rates and count of intestinal microflora were inconsistent with increasing levels of Aurofac supplementation.

The stimulating influence of antibiotics on the growth, mortality and feed utilization of chickens, ducks and turkeys has been reported by numerous workers (Macklin *et al.*, 1952; Scott *et al.*, 1952; Harper and Babcock, 1953; Wisman *et al.*, 1954; Atkinson *et al.*, 1954; Price *et al.*, 1957; Rosenberg *et al.*, 1952; Catalan, 1952; Salinas, 1954; and Alcasid III, 1955). The effect of antibiotics on the microflora of poultry has also been reported by Cook *et al.* (1954) who found that with nine ppm penicillin in the ration of turkey poults, lactobacilli were unchanged in the gizzard and caecum but markedly reduced in the small and large intestines and duodenal loop. These workers reported further that coliforms were unchanged in the caecum but were 10 to 1,000 fold higher in the small and large intestines. Anderson *et al.* (1951) revealed that penicillin enhanced the coliform and lactobacilli counts in the caecum of chicks. Using terramycin at both 100 and 500 grams per ton of ration, Price *et al.* (1957) found a markedly reduced coliform invasion and some influence on the course of the chronic respiratory disease. The same levels with penicillin, however, failed to suppress coliform invasion.

Branion *et al.* (1953) reported that aureomycin reduced the numbers of all groups of microorganisms in the caeca of ducklings. On the other hand, penicillin increased the number of aerobes, anaerobes and coliforms while terramycin increased the number of aerobes, aciduric and coliform types of organisms and decreased the other groups of organisms. Likewise, streptomycin increased the number of aerobes, aciduric, coliform and enterococci types of organisms. These authors further reported that addition of the four

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antibiotics at a level of 25 parts per million to the diet resulted in the lowering of the pH of the cecal contents. March and Biely (1952) observed that aureomycin suppressed lactic acid bacteria and with higher levels there was complete suppression of aerobic and coliform bacteria in the feces.

Investigations made by Elam *et al.* (1951b) showed a different result. Penicillin increased the penicillin-and aureomycin-resistant bacteria and the yeast count. Rosenberg *et al.* (1952) concluded that although the antibiotic significantly stimulated the growth rates there was no reduction in the fecal microflora but enterococci counts were greater in the groups receiving the higher concentrations of terramycin. A similar study made by Cook *et al.* (1952) showed that penicillin increases coliform counts.

The present study was undertaken to determine the effect of different levels of Aurofac on the bacterial count of the small intestines, growth, mortality and feed utilization of New Hampshire and White Leghorn chicks.

MATERIALS AND METHODS

A total of 228 White Leghorn and 181 New Hampshire day-old chicks were used in two trials of eight weeks each, made at different times. There were five lots of New Hampshire and White Leghorn chicks in each trial treated as follows: lot I, basal diet; in the remaining lots Aurofac was added to the basal diet in varying levels as follows: lot II, five per cent; lot III, one per cent; lot IV, two per cent and lot V, four per cent. The composition of the basal diet is given in table 1. The basal ration used in this study

TABLE 1.—*Composition of basal diet*

INGREDIENT	PARTS BY WEIGHT	% CRUDE PROTEIN CONTRIBUTED BY INGREDIENT
Rice bran	15.0	1.92
Ground corn	45.2	4.43
Soybean oil meal	15.0	6.17
Fish meal	10.0	6.20
Molasses	5.0	0.15
Corn gluten feed	5.0	1.15
Ipil-ipil leaf meal	3.0	0.73
Salt	0.5	—
Cod-liver oil	0.3	—
Ground oyster shell	1.0	—
Total	100.0	20.75

had been evaluated by Ranit (1958) and was found to be more nutritionally balanced than the ration used by Alcasid (1955).

Initial and weekly weights, mortality and feed consumption were recorded.

For the determination of bacterial counts of the intestinal content two representative birds from each lot were sacrified. The contents of the small intestines were aseptically removed and placed in test tubes. After a minute of shaking, one ml. of the content was transferred into a vial containing 9.0 ml. of sterile distilled water and the mixture was shaken for one minute. An aliquot of 0.01 ml. was transferred into a clean glass slide and mixed thoroughly with enough amount of Congo Red stain. One square centimeter was made out of this material and then allowed to dry. After air-drying the smear was treated with enough acid-alcohol, allowed to dry and then examined under the oil immersion objective of the calibrated microscope.

In determining the total number of cells per ml. of the content of the small intestine, sixty fields were examined for every smear. The number of individual cell count was multiplied by the microscopic factor (600,000) and the dilution (10).

RESULTS AND DISCUSSION

Effect on growth. The increase in body weight of the S. C. White Leghorn and New Hampshire chickens followed the same trend of increasing level of Aurofac supplementation up to 2.0 per cent (table 2.). At this level, the Leghorns averaged 717.5 grams

TABLE 2.—*Average weight in grams at eight weeks*

PER CENT, AUROFAC	S. C. WHITE LEGHORN	NEW HAMPSHIRE
None	550.2	577.5
0.5	626.2	630.0
1.0	638.2	656.9
2.0	717.5	727.9
4.0	639.4	692.8

and the Hampshires, 727.9 grams. At the 4.0 per cent level, a marked decrease in average weight was obtained. This result indicated a rather low tolerance of both breeds for higher feeding of Aurofac. In all the supplemented lots growth was slightly improved compared to the unsupplemented basal diet. However, weight differences among all the lots were statistically insignificant.

Effect on feed intake and utilization. Table 3 shows the average amount of feed consumed and efficiency of feed utilization. These results show that the 2.0 per cent level of Aurofac supplementation required only 2.65 and 2.48 kilos of feed to produce a kilogram gain in body weight at eight weeks for the Leghorns and Hampshires, respectively. This conversion was quite efficient compared to the other treatments.

TABLE 3.—*Feed intake and conversion*

PER CENT, AUROFAC	WHITE LEGHORN		NEW HAMPSHIRE	
	Consumed (kg.)	Conversion [a]	Consumed (kg.)	Conversion
None	1.73	3.75	1.71	3.50
0.5	1.61	3.20	1.74	3.15
1.0	1.81	3.08	1.84	2.97
2.0	1.70	2.65	1.69	2.48
4.0	1.69	3.10	1.81	2.76

[a] Kilograms feed to produce one kilogram in body weight.

The different levels of Aurofac did not seem to influence feed consumption since the increasing levels of supplementation did not effect a similar trend of increasing feed consumption. Studies by Slinger and Pepper (1954) showed that a supplement of antibiotics increased the feed intake of turkey poults which could be attributed to an effect on intermediary metabolism. The increased growth, therefore, of the chicks used in this study with supplementation of Aurofac up to 2.0 per cent, can not be entirely attributed to increased feed consumption but to an improved utilization of feed nutrients. Evidences from various workers presented by Combs (1956) indicate that antibiotics improve the utilization of protein, enhance the absorption of certain minerals, and increase the blood concentration of certain vitamins.

Effect on bacterial flora. There was a considerable variation in count of bacterial flora in the small intestines of the birds (table 4). The increasing levels of Aurofac supplementation generally caused a decrease in bacterial population with males but not with females of the two breeds. This reaction suggested differences in the kind of bacterial population for both sexes. It is possible that coliform bacteria was predominant in the intestines of females while lactobacilli was of greater concentration in males. Combs (1956) who cited the work of Rhodes *et al.* reported increases in the num-

bers of coliform bacteria in the intestinal tract of chicks fed chlortetracycline but the numbers of lactobacilli were reduced.

TABLE 4.—*Bacterial cell counts per milliliter of intestinal contents* ($N \times 10^6$)

PER CENT, AUROFAC	WHITE LEGHORN		NEW HAMPSHIRE	
	Male	Female	Male	Female
None	15.8	34.55	10.15	8.1
0.5	43.75	43.1	59.7	76.7
1.0	36.8	11.2	55.15	70.7
2.0	20.35	14.1	24.6	21.5
4.0	11.3	24.5	11.7	28.25

While great variation in bacterial counts were obtained in males and females fed varying levels of Aurofac, the differences among treatments and between sexes were insignificant.

Effect on mortality. Even with increasing levels of Aurofac supplementation, the inconsistent mortality rates in all the lots indicated little or no influence of the antibiotic supplement in reducing mortality (table 5). This result is contrary to the findings of Alcasid (1955) who reported that Aurofac lowers mortality in chicks. The failure of Aurofac in reducing mortality even with higher levels of supplementation may be attributed to the nutrient quality of the ration used. Combs (1956) presented evidence from the reports of other workers that antibiotic gave better response when added to a ration less adequate in nutrient composition than from one which is more completely balanced.

TABLE 5.—*Percentage mortality in all the treatments*

PER CENT, AUROFAC	WHITE LEGHORN	NEW HAMPSHIRE
None	11.7	19.1
0.5	21.7	21.7
1.0	20.1	18.6
2.0	18.3	8.6
4.0	27.4	22.5

SUMMARY AND CONCLUSION

Aurofac in levels of 0.5, 1.0, 2.0 and 4.0 per cent was supplemented to a basal ration containing approximately 21.0 per cent protein. Using S. C. White Leghorn and New Hampshire chicks, the

2.0 per cent level of Aurofac supplementation produced the highest average weight and most efficient utilization of feeds at eight weeks of age. The addition of 4.0 per cent Aurofac, however, reduced rate of growth. The increasing level of Aurofac supplementation did not reduce mortality and count of intestinal microflora in both the male and female chicks of both breeds.

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ARTIFICIAL INSEMINATION IN PIGS I. SEMEN COLLECTION BY ELECTRICAL STIMULATION IN BOARS [1]

NESTOR C. VERA CRUZ [2]

In artificially inducing ejaculations by electrical stimulation, the apparatus used was an electro-stimulator designed primarily for use with sheep. It consists of a vibrator unit connected across the primary winding of a centre-tapped transformer that delivers 50 volts from a 6-volt input wet-cell battery, and a bipolar rectal electrode 18 inches long with a terminal pole and three-ring electrodes spaced 2 inches apart.

The rectal electrode is inserted 12 to 16 inches in the rectum of the boar and current is passed between the terminal pole and the 2-inch ring, the current being applied gradually from zero to 30 volts, with stimulations of five to 10 seconds. The electric current used was 60 cycles and the voltage peak which induced ejaculation was 25 to 30 volts.

The resulting ejaculates from 41 stimulations of two boars were of good motility and normal morphology. The average volume of the ejaculates was 99.9 ml. and the semen was characterized by a low proportion of gel-like material (average of 5.0 ml. per ejaculate). Sperm density was high, the average being 403,791,000 per ml. The ejaculate was not extruded in fractions as in natural mating or by the artificial vagina method of collection, and it did not coagulate.

Although no systematic investigation was made on the neural mechanisms involved in ejaculation, it is considered likely that they would be similar to those found by Gunn (1936) in his work with sheep. Ejaculation without erection is affected by stimulation of the nerves in the lumbar region, while stimulation of the nerves in the sacral region causes erection and ejaculation but harmful bodily effects are observed during and after stimulation. These include distressed condition, paralysis of hind limbs and loss of appetite.

The most suitable technique was with the use of the terminal pole and the 2-inch ring electrode and stimulating the lumbar region instead of the sacral region.

Ejaculation could be induced in boars by electrical stimulation and with the proper technique of application. The application of this tech-

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[2] Presently Superintendent of the Los Baños Artificial Breeding Center of the Bureau of Animal Industry at College, Laguna.

nique would permit semen collection under difficult or adverse conditions and from boars that refuse to serve the artificial vagina or are incapacitated to mount due to physical injuries or psychic abnormalities with no harmful effects on the animals.

Electro-ejaculation may be used for the collection of semen in the artificial insemination of pigs or for experimental work on semen.

Adequate experimental and practical work has been done to demonstrate that semen collection from boars could be easily achieved by simple techniques involving the use of the artificial vagina. Several designs of the artificial vagina have been used with success and most of them are only slightly different from the type used for cows, horses and sheep (McKenzie, 1931; Rodolfo, 1934a). Sometimes a bulb is attached to the air line of the apparatus to give pulsations during the course of collection (Wallace, 1949; Glover, 1955).

Recently, Japanese workers (Ito, Niwa, Kudo and Mizuho, 1948) introduced a simple artificial vagina being used in the routine collection of semen in the breeding centers in the Philippines (Montemayor, 1956). This piece of equipment consists of a smooth rubber tube about a millimeter in thickness, 7½ inches long, 1¼ inches in diameter at the proximal end and tapering to 5/16th of an inch in diameter at the distal end. Construction of the tubing is such that both ends are rigid and the middle portion soft. The necessary pressure and thermal condition is applied by the operator's hand.

Fortunately, the boar could also be trained to mount a "dummy" sow (Rodolfo, 1934b; Glover, 1955) which makes the work of collecting semen relatively easy.

However, under difficult conditions that would preclude the use of the artificial vagina in semen collection such as, boars that refuse to mount the "dummy" sow or serve into the artificial vagina or animals that could not mount due to some physical injury, other methods of collection must be developed. In cattle and sheep, a method of semen collection by electrical stimulations of the central nervous system has been perfected.

Electrical stimulation has been shown to induce penile erection and in 1936, Gunn perfected a satisfactory technique of collecting semen from rams with the use of a copper electrode inserted into the rectum and a steel needle electrode placed in the longissimus-dorsi muscle just above the lumbar vertebrae and utilizing a 60-cycle current of about 30 volts.

Since then, various modifications of the apparatus have been made and the technique applied to other species (Bonnadonna, 1938; Lambert and McKenzie, 1940; Terrill, 1940). Laupland and Cassow in 1949 introduced a significant modification of this technique in which the electrical stimulus is introduced through a single rectal bipolar electrode. This method was further used on rams by Ortavant *et al.* (1948), Mata and Cano (1952), Likar and Kamlin (1953), and Dzuik *et al.* (1954a), for application to bulls. Dzuik *et al.* (1954b), have also applied this technique in collecting semen from bulls, rams, boars and goats.

As far as the writer is aware, no work has been done on pigs regarding the use of electro-stimulation for collecting semen except the limited report by Dzuik *et al.* (1954b). These workers reported that they were able to obtain one to eight cc. of semen of good motility from the boar using a 60-cycle current and 12 to 17 volts. The information in their report about the application of this technique to boars is scanty and as the volume of semen they collected was so small (boars normally ejaculate 200 to 250 ml. per copulation) this study was conducted to determine whether or not the principle of electrical stimulation could have practical application.

MATERIALS AND METHODS

Two apparently healthy and normal boars, an eighteen-month-old Canadian Berkshire and a nine-month-old Large White boar were stimulated once a week and sometimes twice depending on the need for semen for experimental purposes.

A portable electrical ejaculator designed specially for sheep was used. It has a vibrator unit which is connected across the primary winding of a centre-tapped transformer that delivers 50 volts from a 6-volt input wet-cell battery. The required voltage of zero to 30 volts is produced by varying the input voltage through potentiometer. This stimulator is housed in a steel case $7\frac{1}{2} \times 5\frac{3}{4} \times 3$ inches.

The electrode consists of a 15-inch copper tubing with an internal diameter of $\frac{1}{4}$ inch. The terminal pole is a brass cap soldered to one end. Holes drilled at 2, 4, and 6 inches provided passage for insulated wires which are connected to 3/16th-inch brass rings which are soldered tightly over the holes. The current is passed through the brass terminal pole and either of the three electrodes spaced 2

inches apart by putting on a switch and turning on a knob to the desired electrode (2, 4, or 6 inches) to use.

The animal is suitably restrained and held on a lateral recumbent position during the operation. Since the general reaction of the body during stimulation is towards extension, the most suitable position of restraint is towards extension. The electrode is inserted into the rectum of the boar to a depth of about 16 inches. Stimulation is started by passing five volts between the terminal brass cap and the 2-inch ring. Duration of this stimulus is from five to 10 seconds after which the current is shut off for a similar duration of time. Then the current is put on again, and the voltage increased to 10 volts by means of a rheostat; this voltage is maintained for another five to 10 seconds and then the current shut-off for another five to 10 seconds. The voltage of subsequent stimulation of the same duration is increased each time by five volts until a maximum of 30 volts is reached, after which the current is reduced to 25 volts. From this time on, application of the current is done by gradually increasing the voltage from nil to 25 volts and decreasing it back to nil within the space of five to 10 seconds. A period of similar duration is allowed before the operation is repeated until enough semen has been obtained or until the boar does not ejaculate any more.

Semen is collected in a wide-mouth sterile bottle previously warmed to 37°C up to 49°C to prevent temperature shock to the spermatozoa. It is then brought immediately to the laboratory where density and motility estimations are made microscopically and where it is stored for other experiments. Density estimations are made with a Fuchs-Rosenthal counting chamber on duplicate samples diluted to 1:800 with formal-saline solution. The average of the two counts is then recorded. Motility of the spermatozoa is assessed by visual methods under the high power of the microscope and scored as follows:

5 = 80%–100% of sperms progressively motile.
4 = 60%– 80% " " " "
3 = 40%– 60% " " " "
2 = 20%– 40% " " " "
1 = 0%– 20% " " " "

Volume, color and odor of the samples are also observed and recorded. Other observations made are the general body reactions of the pigs during the stimulation process, duration of stimulation, and effect on the pigs after ejaculation.

RESULTS AND DISCUSSION

A summary of the data on total volume, volume of gel per ejaculate, density and motility of sperms from 41 ejaculates is given in table 1.

TABLE 1.—*Summary of results of electro-ejaculation in the boar*

BOAR USED	NO. OF COLLECTIONS	TOTAL VOLUME PER EJACULATION (ml)	VOLUME – gel (ml)	VOLUME gel (ml)	DENSITY cell/ml	MOTILITY SCORE
Canadian Berkshire	10	98.5	91.5	7.5	548,660,000	4.9
Large White	31	101.3	98.3	2.6	438,923,000	4.1
Total/Average	41	99.9	94.9	5.0	493,791,000	4.5

Volume of semen collected

Boar semen is chiefly characterized by its large volume in comparison with other species of animals. McKenzie *et al.*, (1938), and other workers are agreed that the average volume of the ejaculate of the boar is about 200 ml. From the data collected on the volume of ejaculate from a Large White and a Canadian Berkshire boar, the average volume collected by electro-stimulation was 99.9 ml. The average of 10 ejaculates of the Canadian Berkshire boar was 98.5 ml. with a range of 30 to 120 ml., while in the Large White Boar (31 ejaculates) the volume ranged from 30 to 176 ml., with a slightly higher average of 101.3 ml. It could be noted here that the volume of semen collected per ejaculate by electrical stimulation is only half as large as the volume normally collected by the use of the artificial vagina.

Volume of gel-like materials

In all the trials, collection of complete ejaculates was insured by following the same procedure throughout which was to stop stimulation and collection as soon as the boars gave out a watery and clear fluid which, upon examination, contained very few or no sperm cells. The smaller volume of ejaculate collected by this method could be explained by the complete absence or presence in small proportions of the gel portion charactistic of "normal" ejaculates and perhaps a reduced secretion by the other accessory organs, as McKenzie, Miller and Baughes (1938) reported that the glands responsible for gel secretion are the seminal vesicles, Couper's gland and possibly the urethra.

In a study of the contribution of accessory organs to the semen of the pig, McKenzie *et al.* (1938) by systematic removal of the accessory glands, found that the prostrate and urethral glands contribute 62 per cent, Couper's gland 15 per cent, seminal vesicles 20 per cent and the epididymis 3 per cent to the whole ejaculate. These workers also report that the gelatinous tapioca-like material which is a characteristic constituent of "normal" ejaculates constitutes from 30 to 40 per cent of the whole ejaculate.

The result of this study however, shows that only a very small proportion of the semen collected by electrical stimulation is gel. In most ejaculates, especially from the Large White Boar, gelatinous material was completely absent. The average gel content of the 41 ejaculates studied was 5.0 ml. or 5 per cent of the total ejaculate, ranging from zero to 30 ml. The gel content of the semen collected from the Canadian Berkshire boar was 7.5 ml. and from the Large White boar, 2.5 ml. The difference is very slight and may be explained by the fewer number of collections from the Berkshire boar and perhaps to differences in individuality and age of the two subjects. The semen in all cases did not coagulate.

The occurrence of the almost gel-free ejaculate is significant as these gelatinous materials are normally filtered from the semen used in artificial insemination. Although Johari (1956) suggests that the gel of boar semen has other functions besides acting as a plugging material, there has been no evidence that it is essential for normal fertilization and impregnation. It could therefore, be considered an advantage of electrically-ejaculated semen that it contains only a very small proportion of this gelatinous material.

Semen concentration

Again, contrary to the expected low density of boar semen, which is on the average of the order of 100,000,000 to 200,000,000 per ml., the density found in the ejaculates in this experiment was fairly high to the extent that some ejaculates show swirling motion when seen through the microscope. Ejaculates from the Berkshire boar ranged in density from 217,600,000 to 1,024,000,000 sperms per ml. with an average of 548,660,000 sperms per ml., while those from the Large White boar ranged from 83,000,000 to 900,000,000 sperm cells per ml., averaging 438,923,000 sperms per ml. The average of all ejaculates was 493,791,000 spermatozoa per ml. which is more than twice the normal density. It is of interest to note that although the

volume of semen collected by electrical stimulation is smaller, the density of sperm cells is higher: thus the total number of spermatozoa in the whole ejaculate would be approximately the same as with ejaculates from natural matings or those collected by the use of the artificial vagina.

Motility of sperms

Motility of the sperms collected in this study as assessed by the usual method of visual-microscopic examination was good. The majority of ejaculates gave a perfect score of five (80 to 100 per cent progressively motile sperms) and only on three occasions were the sperms immotile on collection. Examination of these ejaculates revealed a high percentage of spermatozoa with protoplasmic droplets. A study of the dates and interval of collection reveals that the ejaculates of poor or dead spermatozoa are those collected on the third day within a week; collections being of two-day intervals. The frequency of collection, however, does not show any direct relationship to volume of ejaculate or sperm density. This study suggests that collection by electrical stimulation could be made at weekly intervals or two collections could be made within a week. This observation is in accord with the recommendations of Polge (1956) based on information from studies by Rodolfo (1934a) and McKenzie *et al.* (1938) viz., that boars in artificial insemination centers could be used for semen collection twice to thrice weekly and must produce 500 ml. of semen with an average sperm concentration of 1×10^5 per ml. or 5×10^{10} sperms per week.

Duration and pattern of ejaculation

Ejaculation is a relatively long process in pigs. It takes about five to 30 minutes to complete one ejaculation. Several investigators also report that ejaculation in the boar follows a pattern and semen could be fractionated into three dictinct portions: the pre-sperm, sperm-rich and the post-sperm fractions (McKenzie, 1938; Glover, 1955; Rudolfo, 1934a). These workers observed that the boar thrusts its penis vigorously into the artificial vagina and this thrusting motion is generally maintained for a few minutes during which time a clear, sticky fluid containing gel-like materials is emitted. When the vigorous thrusting motion ceases, the volume of thick,creamy semen ejaculated is increased. Finally, towards the end of ejaculation, a large proportion of gel-like material is observed.

In the ejaculates collected by electro-stimulation, this pattern of ejaculation was not observed. The appearance and consistency of the semen was the same throughout the process except on certain occasions when a few pellets of gel were first extruded. The entire process of stimulation and collection by the electric stimulation technique took from eight to 11 minutes. The average duration of the 41 ejaculates was calculated to be 9.5 minutes. There does not seem to be any direct relationship between duration of stimulation and volume of semen or sperm density.

Color and odor of ejaculates

The typical ejaculum produced by electrical stimulation is grayish to creamy white in color and has no odor except for a little fishy smell. This is in contrast to occasional abnormal ejaculates which are tinted amber to light brown, contaminated with cellular debris from the bladder, and which smell strongly of urine. The spermatozoa in such ejaculates are generally short-lived, motility lasting for 15 minutes at most. This cessation of motility may perhaps be due to the urine contamination in the semen, for when semen of this kind is centrifuged, the plasma separated and discarded, and the packed cells rediluted with glycine-egg yolk diluters, the motility is restored. Gunn (1936) also observed the same phenomenon in ram semen collected by electro-ejaculation and he reports that ewes inseminated with this kind of semen may conceive.

Non-contamination of semen could be insured by allowing the urine to flow out uncollected during the first few applications of the stimulus.

Reaction of the animals to the electrical stimulus

Ejaculation is generally considered to be essentially a spinal-reflex action, for according to Marshall (1922), ejaculation is a result of sexual emotions which are cerebral in origin but it may occur even after transection of the cord above the lumbar region. As cited by Gunn (1936), Anderson and Langley (1895) found that erection is caused by stimulation of the cord via the sacral nerves to the pelvic nerves causing vasodilatation and inhibition of the unstriped muscles of the penis or to the pudendal nerves causing contraction of the ischiocavernosus, bulbo-cavernosus and urethral muscles, and that reverse effects follow stimulation of the lumbar nerves, the greatest effects being produced by stimulation of the third to the fifth lumbar

vertebrae in cats and rabbits and the first to fourth lumbar vertebrae in goats.

The expulsion of semen is effected by contraction of the urethral bulbo-cavernosus and the ischiocavernosus muscles and the whole process of ejaculation is really a series of expulsive consecutive actions of the urinogenital organs beginning with the vas efferentia of the testes, through the epididymis and vas deferens which contracts almost simultaneously with the seminal vesicles to the ejaculatory duct, continuing to the urethra where prostratic secretion is expressed by contraction of the surrounding muscles (Marshall, 1922).

It is apparent from the reports of many researchers that the nerves concerned with ejaculation do not arise from the same place as those concerned with erection. Gunn (1936), reporting on his experiments on the collection of semen from rams by electrical ejaculation, stated that stimulations of the sacral part of the cord or its nerves (essentially the pelvic autonomic outflow) produces erection, expression of the stored secretion of the prostrate and possibly also that of other glands, and ejaculation which may not be necessarily accompanied by emission of semen, while stimulation of the lumbar part of the cord or its nerves (essentially the lumbar sympathetic outflow) results in retraction of the penis by causing contraction of the retractor penis muscle innervated from the cranial source in the lumbar region or caudal source in the thoracic region, diminution of the volume of the penis, contraction of the musculature of the secretory and storage sex regions and secretion by the accessory sex glands.

An initial experiment on the Canadian Berkshire boar was repeated with the Large White boar and results in the two animals were essentially the same. Using the technique of stimulation previously described, the rectal electrode using the 2-inch pole, was inserted into the rectum at two depths, six inches (sacral) and 12 inches (lumbar). Erection and ejaculation were effected by inserting the electrode four inches in the rectum but the animals were greatly distressed during and even after stimulation. They were observed to be paralyzed in the hind quarters, and unable to stand until three to 24 hours after ejaculation. On the other hand, in the trials with 12 inches inserted in the rectum, ejaculation was effected but no erection could be induced. The animals, however, did not show any distress during or after the stimulation.

It was therefore decided that the electrode be inserted to a distance of 14 inches in the case of the older and larger Berkshire boar and 12 inches for the younger, smaller Large White boar. This latter depth was, however, gradually increased to 14 inches as the animal grew bigger. Production of erection was decided against owing to the harmful effect of stimulation of the sacral region on the boars. Unfortunately, this meant forsaking collection of a virtually sterile semen. However, the penis was held inside the collecting bottle, utmost care being taken to clean the region around the prepuce before collection.

This procedure was adopted throughout the course of the main series of collections. However, the poles used were again varied from two to four inches. Table 1 showed that when the current was passed between the terminal pole and the 4-inch ring, paralysis of the hind quarters resulted in all cases with both boars; moreover, the animals were distressed during stimulation. This paralyzed condition lasted for three to five hours after which loss of appetite for one to two days was noted.

With the use of the terminal pole and the 2-inch brass ring, no distressed condition or paralysis was observed. On first application of the stimulus, a general tetanic contraction of all the muscles of the body, particularly the extensors, was observed and respiration was held in abeyance. The pupils of the eye dilated momentarily for a few seconds. At each stimulation, the eyelids were noticed to close, the ears became erect, and the testes and scrotum were drawn up due to contraction of the dartos and cremaster muscles.

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NOTE: OCCURRENCE OF POWDERY MILDEW OF CITRUS IN THE PHILIPPINES

WITH TWO TEXT FIGURES

No report on the powdery mildew of citrus has hitherto been published in the Philippines. In the summer of 1959, however, a striking occurrence of the disease was observed by the writers in the orchard of Professor M. V. Jarmin at Kapatagan, Los Baños, Laguna, where no single tree was free of infection. The disease was also noted in the Bicol region on June 10, 1959, in the 14-hectare citrus orchard of Mr. Nicanor S. Clasio, provincial agriculturist of Camarines Sur. The disease has also been recently reported occurring in serious proportion in the orchard of Dr. Jose R. Katigbak in Lipa City, and in other citrus orchards in the province of Batangas. In these orchards the disease was observed in *Citrus nobilis* Lour. cv. 'Laddu'. Apparently, this citrus disease which is posing a potential threat to the citrus industry of this country still remains unrecognized and uncontrolled by many of the citrus growers. The present paper records the occurrence, describes the symptoms, presents the etiology and offers possible control measures for the disease.

APPEARANCE OF THE DISEASE

The symptoms of the disease are similar to those described by Fawcett.[1] Young shoots and twigs are most commonly and severely affected. Suckers which are produced immediately after the rains and are left to develop from the base through the center of the trees are generally affected (fig. 1). White patches of powdery masses more frequently noted on the upper surface of the leaves, are the most discernible feature (fig. 2). The fungus does not spread in a perfectly circular manner, but hyphae may be seen to radiate from the original center of infection. The mycelium which has a rather coarse appearance is not dense. The leaf tissues beneath the fungus mycelium are at first a dark watery green and, as the disease progresses, they lose their normal color and turn yellow.

[1] H. S. Fawcett, "Citrus Diseases and Their Control," McGraw-Hill Book Co., Inc., New York and London, 1936, xv + 656 p.

Fig. 1.—Powdery mildew on *Citrus nobilis* Lour. cv. 'Laddu'. At left, defoliated and partially dried water sprouts. At right, crinkled leaves due to infection. (*Picture taken by the Photographic Division*).

Fig. 2.—Naturally infected leaves of *C. nobilis* cv. 'Laddu' showing the recognizable sign of powdery mildew. Whitish areas represent the superficial fungous structures. (*Picture taken by the Photographic Division*).

ETIOLOGY

Infection on the young leaves spreads rapidly and covers a large portion of the leaf. Such leaves shrivel, dry and adhere to the twigs. If the shoot is very succulent, the hyphae may spread from the leaves down onto the twigs for several inches. Older leaves show no shriveling but a buckling of the tissues and malformation follows.

The morphological characteristics of the mycelium, spores and conidiophores which compose the powdery growth covering the infected leaves and twigs conform to those described for *Oidium tingitanium* Carter. The spore size however, varies depending upon the host and environment.[2] The spores of the citrus mildew organism in the Philippines vary from 21.65 μ to 43.29 μ by 11.65 μ to 19.98 μ. The average length and width of spores are 33.6 μ and 14.5 μ, respectively.

CONTROL MEASURES

There is a paucity of information on the control of powdery mildew of citrus. Where infection is likely to cause tremendous damage, spraying with Bordeaux mixture or dusting with sulfur [3] will help minimize losses due to the disease. A weekly schedule of lime-sulfur spray during damp warm weather may satisfactorily control the disease.[4]

Water sprouts which grow from the center of the trees immediately after the rains should be pruned and burned since they are the most susceptible parts of the plant from which secondary spread usually originates.

—B. P. Tamayo and A. N. Pordesino

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[2] C. N. Carter, *Phytopathology*, **5**, 193-196 (1915).
[3] S. Sundararaman, *Report Dept. Agric.*, Madras, 1927-1928, pp. 355-372 (1928).
[4] W. C. Lester-Smith, *Phytopathology* **22**, 870 (1932).

NOTE: DRY BEAN PRODUCTION OF PROMISING BUSH LIMA BEANS

Lima beans are very popular because of their value as human food and the comparative ease with which they can be grown. In addition, they aid in maintaining soil productivity if the crops are handled in the proper way.

The native lima beans are tall and viny requiring poles or stakes on which to climb. These poles increase cost of production and are rather inconvenient in commercial planting. Foreign bush lima beans are essentially bushy in growth habit and have been observed to be early maturing and generally more productive than the native varieties. They fit better in commercial cropping and crop rotation programs.

This study was conducted to test six varieties of bush lima beans for their dry bean producing capacities under Philippine conditions in the experimental field and laboratory of the Vegetable Crops Section, Department of Agronomy, from January to May, 1959.

A randomized complete block design with four replications was used. Sixty kilograms per hectare each of phosphorus and potassium and 45 kilograms of nitrogen per hectare were applied. Nitrogen was applied as ammonium sulfate (20 per cent N) and potassium as muriate of potash (60 per cent K_2O).

Six 6-meter rows were used. Furrows one meter apart were laid out with a native plow and fertilizers were applied in band at the bottom of the furrow. Three seeds were planted in hills 30 centimeters apart within the row.

The plants were pulled out when a great majority of the pods matured.

The rainfall during the growing season was unevenly distributed. Ample amounts of irrigation water were applied during the periods of low rainfall to keep the soil moisture favorable for the growth of the plants. On the other hand the prevailing temperatures during the same period were relatively high; the weekly temperatures ranged from a minimum of 17.6°C in February to a maxi-

mum of 37.2°C in May, whereas, the average monthly temperatures gradually increased from 25.2°C in January to 29.0°C in May.

Yield of dry beans. Differences in yield between some of the varieties were very marked. Of the six varieties tested, three were promising, the differences being significant at the one per cent level. Habas lima was the best yielder. Differences between Habas and the other varieties were highly significant. U.S. 255 and Baby Fordhook were both significantly better than Burpee Improved which gave the lowest yield of 429.75 grams per 6-meter row plot. No significant differences were found among the U.S. 255, Baby Fordhook, Triumph and Nemagreen varieties; however, the first two varieties tended to give higher yields than the latter ones.

Weight of seed. The weight of 20 seeds differed markedly between some of the varieties. The weight of seeds of Burpee Improved significant differed from the rest of the varieties at the one per cent level. The differences between Habas and both U.S. 255 and Nemagreen were not significant.

Correlation coefficient. Analysis indicated that there was no direct relationship between weight of seed and yield. Burpee Improved and Nemagreen had the heaviest and the highest weight of seed, respectively.

Growth and blooming habits. All the tested lima beans tended to ripen their pods while still retaining their foliage. Except Burpee Improved, all the varieties tended to grow and flower especially when soil moisture was kept favorable for plant growth.

Burpee Improved showed a tendency to cease growing and flowering after the pods ripened. It showed a weakly twining behavior indicating that it was intermediate between the bush and pole types of lima beans.

Abscission of flowers. It was observed that there was a moderately excessive drop of blossoms following two or more days of excessively hot weather especially from varieties Nemagreen, Triumph, Baby Fordhook and U.S. 255. The super-optimum temperatures that prevailed during the growing period seemed to have provided a good test for varieties in respect to pod setting and an opportunity to observe heat-resistant varieties.

—*Andres F. Celestino*

BOOK REVIEW: HANNA'S HANDBOOK OF AGRICULTURAL CHEMICALS

The book is a valuable reference not only to users and dealers of agricultural chemicals but also to other people in the field of agriculture and forestry, such as teachers, researchers, technicians, extension agents and fieldmen. In this handbook descriptions and uses of over 1000 commercially available agricultural chemicals have been assembled together in one volume of convenient size for ready reference. The author, Lester W. Hanna, is entomologist for the Western Region of the Birds' Eye Division of General Goods Corporation. His 30-year experience in the practice of his profession under various capacities including more than seven years as forest entomologist of the U.S. Forest Service and his contact with various workers in his field of interest provided the background in preparing his handbook.

The handbook contains 489 pages, divided into 17 chapters. The first chapter deals with pesticide toxicity, registration and residue tolerances. It includes the complete list of official chemical tolerances recently issued by the U.S. Federal Food and Drug Administration. Other chapters contain data on trivial code of entomology, entomological training of youth (4-H Club work) and weather and compatibility charts.

An enumeration of the chapters will enable one to determine the scope of the book. These chapters include Pesticide toxicity, registration and residue tolerances; Public Law 518 (Miller Bill); Trivial Code of entomological terms; Official antidotes, emergency treatments, miscellaneous drugs and clinical laboratories; Safety; Formulae, figures, measures and tables; Entomological training of youth; Fertilizers; Fumigants; Fungicides; Herbicides; Insecticides; Livestock chemicals; Rodenticides; Miscellaneous—Aerosol propellants, frost protectors, electronic devices, diseases, pollen, hybridization, food radiation, biological insect control, forest entomology, fire protection, etc.

—G. V. Viado

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